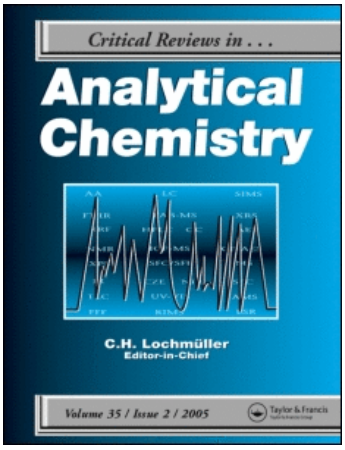

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Chromatographic Methods for the Analysis of Polycyclic Aromatic Hydrocarbons in Water Systems

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CHROMATOGRAPHIC METHODS FOR THE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN WATER SYSTEMS

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I. INTRODUCTION

A number of analytical techniques have become available for the analysis of polycyclic aromatic hydrocarbons (PAHs) in water systems. The concentration of PAHs in water systems ranges from the ppt(ng/ℓ) to the ppm(mg/ℓ) level so that a preconcentration step prior to analysis is always required. In samples from heavily polluted waters, a cleanup step to separate the PAHs from the other organic solutes is necessary. The PAHs most commonly encountered in water systems are listed in Table 1. Two approaches are possible: (1) an initial chromatographic separation followed by identification, or (2) a total analysis by spectroscopy. In general the complexity of the sample limits the advantages of the one-step spectroscopic approach. The details of spectroscopic analysis as applied to PAHs will be discussed in a later publication.

Smith et al.[1] have reviewed the procedures for sampling, preconcentration, and cleanup of PAHs from water systems. Generally, the cleanup involves the isolation of the PAH fraction with thin layer chromatography (TLC) or column chromatography.

The problem of separating and analyzing a class of compounds that contains no functional groups and has a large number of structural isomers is a difficult one. All the chromatographic methods have been applied to this problem with varying degrees of success. Cleanup by column chromatography has been used prior to TLC in a number of procedures. Gas chromatography (GC) using a number of supports and stationary phases, glass capillary gas chromatography (GC^2), and high performance liquid chromatography (HPLC) have all been used for PAH analyses.

When the PAHs have been separated, a number of methods for detecting and measuring the amount of PAHs present are available. UV or fluorescence detection are convenient methods subsequent to TLC or HPLC separation. Flame ionization detection, or mass spectrometry are generally used with GC or GC^2. Photoionization and gas phase fluorescence detection methods are being developed.

The factors which must be considered in comparing and evaluating the various analytical techniques are (1) separation efficiency, (2) lower limits of detection, and (3) ease and speed of analysis. Separation efficiency should include complete isolation of the PAHs from the other organic pollutants and the resolution of the PAH fraction into its various components. Structural isomers of PAHs often vary in carcinogenic activity. For example, benzo(*a*)pyrene (B(*a*)P) is an extremely active carcinogen whereas benzo(e)

Table 1
PAH AND OTHER AROMATIC HYDROCARBONS

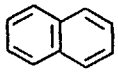
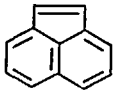
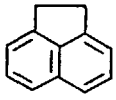
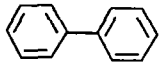
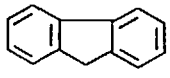
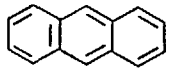
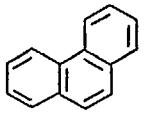
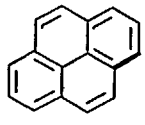
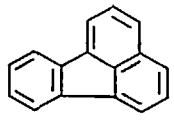
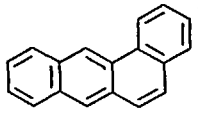
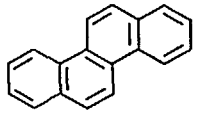
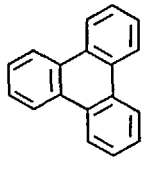
Structure	1957 IUPAC name	Other names	Molecular weight	Common literature abbreviation (if any)
	Naphthalene	—	128	—
	Acenaphthylene	—	152	—
	Acenaphthene	—	154	—
	Biphenyl	—	154	—
	Fluorene	—	166	—
	Anthracene	—	178	—
	Phenanthrene	—	178	—
	Pyrene	—	202	—
	Fluoranthene	—	202	—
	Benz(a)anthracene	1,2-Benzanthracene	228	B(a)A
	Chrysene	—	228	—
	Triphenylene	—	228	—

Table 1 (continued)
PAH AND OTHER AROMATIC HYDROCARBONS

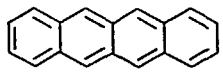
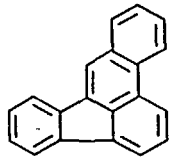
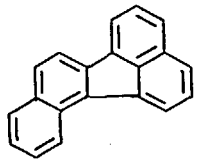
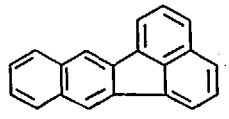
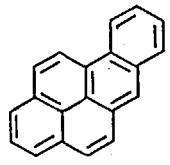
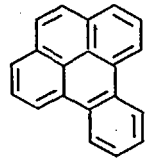
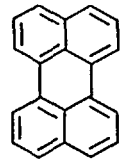
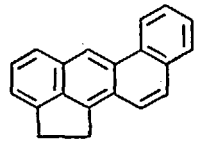
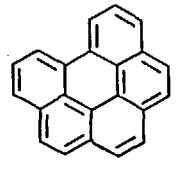
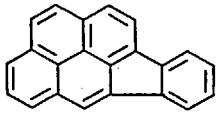
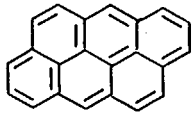
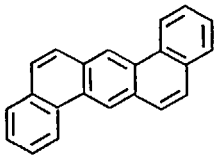
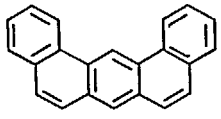
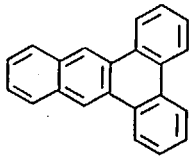
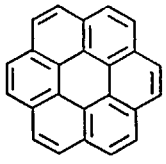
Structure	1957 IUPAC name	Other names	Molecular weight	Common literature abbreviation (if any)
	Naphthacene	Tetracene 3,4-benzanthracene	228	—
	Benzo(b)fluoranthene	3,4-Benzfluoranthene	252	B(b)F
	Benzo(j)fluoranthene	10,11-Benzfluoranthene	252	B(j)F
	Benzo(k)fluoranthene	11,12-Benzfluoranthene	252	B(k)F
	Benzo(a)pyrene	3,4-Benzopyrene	252	B(a)P
	Benzo(e)pyrene	1,2-Benzopyrene	252	B(e)P
	Perylene	—	252	—
	Cholanthrene	—	254	—
	Benzo(ghi)perylene	1,12-Benzperylene	276	B(ghi)P

Table 1 (continued)
PAH AND OTHER AROMATIC HYDROCARBONS

Structure	1957 IUPAC name	Other names	Molecular weight	Common literature abbreviation (if any)
	Indeno(1,2,3-cd)pyrene	*o*-Phenylenepyrene	276	IP
	Anthanthrene	—	276	—
	Dibenz(a,h)anthracene	1,2,5,6,-Dibenzanthracene	278	D(a,h)A
	Dibenz(a,j)anthracene	1,2,7,8-Dibenzanthracene	278	D(a,j)A
	Dibenz(a,c)anthracene	1,2,3,4-Dibenzanthracene	278	D(a,c)A
	Coronene	—	300	—

pyrene (B(e)P) is relatively inactive; dibenz(a,h)anthracene (D(a,h)A) is highly carcinogenic, while other dibenzanthracenes are not. The lower limits of detection must be sufficient to resolve and identify the PAH isomers. Although time considerations may be significant to some applications, in general they are secondary to good separation and detection. The applicability of the analytical scheme to performance by technicians must also be considered.

II. FRACTIONATION AND CLEANUP PROCEDURES

A. Introduction

The PAHs which are found in water can originate from many different sources, e.g., petroleum spills, sewage, industrial wastes, and washout from atmospheric pollution. Generally the PAHs are a small fraction of the total dissolved organic carbon which

includes aliphatic, olefinic and cyclic hydrocarbons, simple aromatics, organic acids, esters, and bases — as well as oxygen, nitrogen, and sulfur heterocycles. A number of methods have been proposed to isolate the PAHs and simple PAH derivatives. These methods can often involve solvent extraction and gel filtration techniques which were presented earlier.[1] This review deals with column and the thin layer chromatographic methods.

B. Thin Layer Chromatographic (TLC) Methods

TLC separation methods can be carried out relatively simply. Glass plates (20 × 20 cm) are frequently used as supports. Alumina and silica gel are the common stationary phases, although the use of acetylated cellulose has been investigated. The residual water content of the support which can affect the separation efficiency of the adsorbent is a primary concern when using alumina or silica. A number of high-temperature activation procedures have been developed to reduce the water content of alumina and silica to a "zero" level. Some workers have found that the addition of 2 to 3% water leads to better fractionation and less tailing. Regardless of the water content of the adsorbent, it is important to adopt a constant activation procedure to ensure reproducible results.

The choice of the solvent system to serve as the mobile phase depends on the type of adsorbent and its degree of activation. "Dry" nonpolar solvents such as hexane or pentane are used with completely deactivated as well as partially deactivated alumina and silica. Polar mobile phases such as methanol, acetonitrile, and water are used with nonpolar adsorbents such as acetylated cellulose.

Coating of the stationary phase onto the support is important if reproducible R_F values and quantitative analytical results are to be obtained. Manual and automatic applicators are available for coating the TLC plates. The spotting of the sample is performed with a calibrated microsyringe or micropipette or with an automatic plate spotter. Volumes of 1 to 25 $\mu\ell$ can be delivered with random errors of 1% or less.[2] The methods of development for cleanup TLC procedures generally involve one-dimensional developments, since only an isolated PAH fraction is desired.

Nielsen[3] employed a TLC prefractionation procedure on extracts from gasoline and lubricating oils. The TLC plates (20 × 20 cm, 0.25 mm thick, Macherey-Nagel®SIL G-25 HR) were activated for 1 hr at 100° C. The extract and a standard mixture of PAHs were applied as a 6- to 8-mm wide band on the plate. Ascending one-dimensional development in the dark with hexane was followed by development with a 1:1 toluene-cyclohexane mixture. The hexane development was used to minimize possible overloading by a high content of nonvolatile, nonpolar compounds. The PAH fraction was one of six bands identified and was eluted from the silica with two 3-mℓ portions of diethyl ether and concentrated to 100 $\mu\ell$ under a dry nitrogen stream.

Lankmayr and Muller[4] employed a similar procedure to isolate the PAH fraction from other organics that were extracted from an environmental dust sample. Their method can be applied to aqueous sample extracts since the PAHs studied are also commonly found in water. Commercially available 20 × 20 cm SI 60 TLC plates with 0.25-mm thick layers were used (Merck, Darmstadt, G.F.R.). No activation procedures were given. A 5-cm line from an 800 $\mu\ell$ extract was applied to half of the plate; a test mixture of PAHs consisting of benz(a)anthracene (B(a)A), B(*a*)P, perylene, benzo(k)fluoranthene (B(k)F), D(a,h)A, indeno[1,2,3-cd]pyrene (IP), and coronene was applied to the other half. Development was carried out with a cyclohexane-benzene (1:5) mixture. All of the PAHs were found in a small R_F region between 0.65 and 0.75. Less polar compounds, e.g., aliphatics, were found near R_F values of 1.0, and the polar fraction consisting of nitrogen, oxygen, and sulfur heterocycles was found at low R_F values near the origin.

The aromatic PAH zone was identified under UV light of 254 nm and removed from

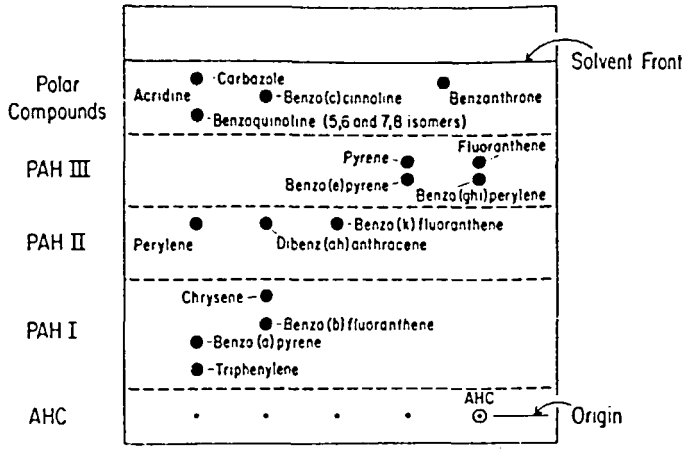


FIGURE 1. Thin-layer chromatogram showing the separation of the PAHs, aliphatic hydrocarbons, and other polar aromatic compounds into different regions of the plate.

the plate into a small test tube. The PAHs were eluted from the silica in an ultrasonic bath for 5 min with cyclohexane. Recovery factors obtained for all PAHs were close to the 90% reported for B(a)P.

Daisey and Leyko[5] isolated a PAH fraction from other organics extracted from airborne particulate matter. A 20 × 20 cm TLC plate coated with 20% acetylated cellulose was spotted 2.0 cm from the bottom at 1.0 cm intervals using a 5.0 $\mu\ell$ disposable capillary pipette with a Wiretrol® dispenser (0.1 to 0.3 mg per spot). A standard mixture of PAHs was also spotted on the plate. After development with *n*-propanol-acetone-water (2:1:1 v/v/v) in a sandwich chamber to a height of 15 cm (approximately 2 hr), the chromatogram was removed, dried, and viewed under a long wavelength UV light. The resulting PAH fraction was spread through an R_F region of approximately 0.15 to 0.80, while the aliphatic hydrocarbons were found at the origin and polar compounds at the solvent front. Figure 1 illustrates these results. The PAHs were found in three distinct bands as shown and are removed from the TLC plates by elution with 40% diethyl ether in cyclohexane (by volume). Recoveries from this step are shown in Table 2, and range from 71% for B(a)A to 101% for benzo(ghi)perylene B(ghi)P. Standard deviations range from 5 to 20% for each PAH studied.

C. Column Chromatographic Methods

Commercial chromatographic columns of various sizes are available; home-made columns from burettes, glass or polyethylene tubing, are also used for column chromatography. The same adsorbents and mobile phases used in TLC are also used in column chromatography. The bands of interest can either be collected as they elute from the column or can be sliced off the column by stopping the mobile phase flow once a desired separation is attained. Since standards cannot be run simultaneously with the sample, standard runs closely duplicating the sample run are required.

Gearing et al.[6] have investigated a column chromatographic technique for isolating the aromatic and aliphatic hydrocarbon classes commonly found in aqueous sediment samples. A standard mixture was studied before application of the method to environmental samples. The standards included n-alkanes with 16, 18, 24, 28, and 32 carbons, pristane, unsaturated paraffins and aromatics, including anthracene and phenanthrene.

Table 2
RECOVERIES OF PAH FROM 20% ACETYLATED CELLULOSE WITH 40% DIETHYL ETHER IN CYCLOHEXANE

Compound	Recovery (%)
Benz(a)anthracene	71 ± 8
Benzo(a)pyrene	90 ± 5
Benzo(e)pyrene	72 ± 5
Benzo(ghi)perylene	101 ± 22
Chrysene	88 ± 9
Dibenz(a,h)anthracene	82 ± 5
Fluoranthene	86 ± 9
Perylene	90 ± 4
Pyrene	89 ± 10

Silica gel and neutral alumina (Woelm) were activated by overnight heating at 200° C. Deactivation was accomplished by mixing the stationary phase with an appropriate volume of water. All columns had an adsorbent to sample ratio greater than 100 and were prewashed with hexane before use. The columns were prepared with varying proportions of silica gel under alumina, (v/v), with different degrees of activation. A 3:2, silica gel-alumina column, 5% deactivated, and a 4:1 activity grade 1 column had a low fractionation efficiency. A column made up of 2:1 silica gel to alumina (both activity grade 1) was found to be the most efficient. After the sample was placed on the column, the aliphatic fraction was eluted with two column volumes of hexane, and the aromatic fraction was eluted with two column volumes of benzene.

The technique was applied to sediment samples from Narragansett Bay. Fractionation reproducibility was determined by GC analysis of the aromatic and aliphatic fractions obtained from replicate column chromatography runs on sediment extracts. The relative standard deviation was 12% for the aliphatic fraction and 27.5% for the aromatics on the basis of 12 runs. Preliminary intralaboratory reproducibility studies gave relative standard deviations of less than or equal to 25%. Gearing et al.[6] found that analogous TLC procedures with identical stationary and mobile phases produced results which were not significantly different.

Giger and Schaffner[7] have developed a procedure for isolating PAHs from environmental samples utilizing gel permeation and adsorption chromatography (Figure 2). After the removal of elemental sulfur from the methylene chloride extract by percolation through a column of activated copper, the extracts were carefully evaporated to dryness. The residue was redissolved in 2 mℓ of (1:1) benzene-methanol and subjected to an initial fractionation on Sephadex® LH-20. For this purpose, a 50 × 1.6 cm glass column was packed with a slurry of 20 g of Sephadex® LH-20 in (1:1) benzene-methanol. The same solvent blend was used to elute two 50-mℓ fractions and a third 50-mℓ portion served to flush the column before eventual reuse. The fractionation on Sephadex® LH-20 proceeded by a combination of exclusion and adsorption processes and was favorably characterized by its speed, high column capacity, and the possibility of column reusage. The two resulting 50-mℓ fractions were again carefully evaporated to dryness (rotary evaporator, reduced pressure, about 30° C), and redissolved in 1 mℓ of *n*-pentane. These fractions were applied to silica gel columns (1 cm I.D., 10 mℓ bed volume); 25 mℓ of pentane and 25 mℓ of methylene chloride as mobile phases resulted in two fractions of low and medium polarity, respectively. Flow rates through the column were

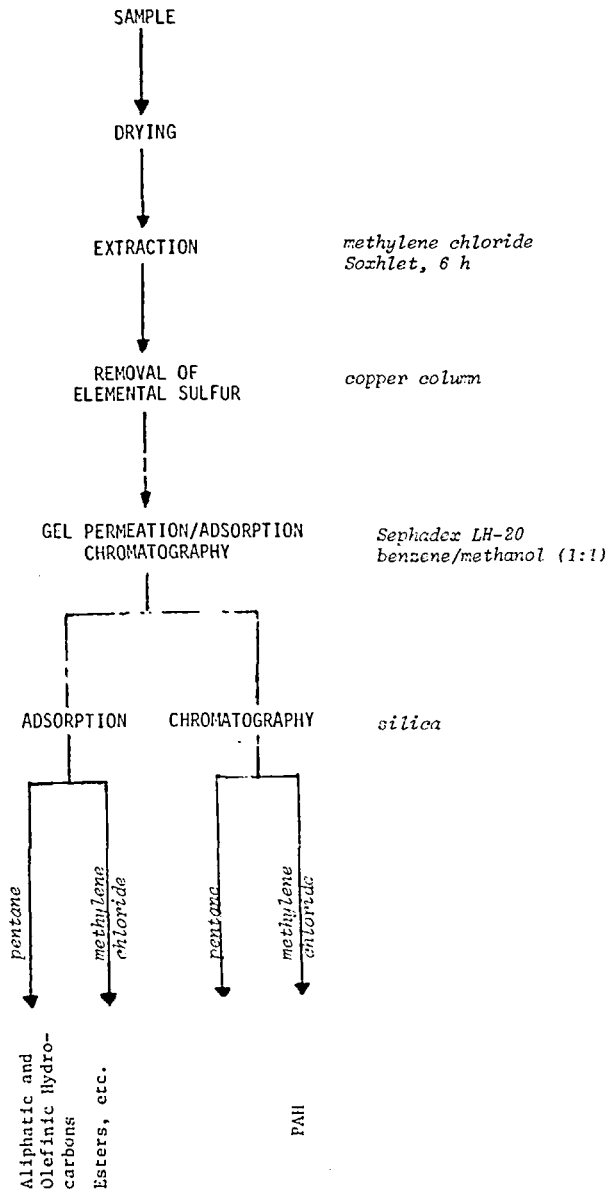


FIGURE 2. Procedure for the isolation of PAHs from other components in environmental samples.

about 2 mℓ/min. The cutpoints of these fractionation steps were established beforehand with naphthalene and coronene standards.

This separation scheme provided two sufficiently pure concentrates: aliphatic and olefinic hydrocarbons in the pentane eluate of the initial Sephadex® fraction and PAHs in the methylene chloride fraction of the material which had been more strongly retained on the Sephadex column®, i.e., the latter Sephadex® fraction. This cleanup scheme is an improvement of an earlier procedure developed by Giger and Blumer.[8]

In 1971, the World Health Organization (WHO) defined the maximum permissible concentration limit of 200 ng/ℓ for the total concentration of six PAHs in drinking

water. The six regulated PAHs are fluoranthene, benzo(b)fluoranthene(B(b)F), B(k)F, B(*a*)P, B(ghi)P, and IP. Borneff[9] has devised a TLC analytical procedure for determining the concentrations in water of the six PAHs following a cleanup column chromatography step. A small glass column (about 6 mm I.D. and 100 mm long) was packed with 0.5 g of alumina, basic, activity II (according to Brockmann). The column was rinsed with 1 mℓ of cyclohexane, and then the sample extract was added to the column. The sample extract flask was rinsed with 0.5 mℓ of cyclohexane which was also added to the column. The resulting eluate was discarded. The development was accomplished by addition of 0.5 mℓ of cyclohexane and 3.0 mℓ of a cyclohexane-to-benzene mixture (1:1). The eluates which contained the PAHs were saved for subsequent volume reduction. It was suggested by Borneff that this procedure be tested with a number of reference standards to establish optimum conditions.

Dunn[10] has developed a procedure for determining B(*a*)P concentrations in marine organisms and sediments which employs an initial column chromatography cleanup step. A column packing of 30 g of Florisil® (60 to 100 mesh) from Matheson Coleman & Bell covered with 60 g of sodium sulfate was prepared in a glass column (4 × 40 cm) with a coarse fritted glass disk. The Florisil® was pretreated by thoroughly washing with distilled water and methanol under suction. Upon the removal of excess methanol, the adsorbent was heated in vacuo at 60°C and then activated at 250°C for 18 hr. Partial deactivation was carried out after cooling by the addition of 2% water. The column was pretreated with 100 mℓ of isooctane; then the sample which had been extracted into isooctane was added. Two 100-mℓ portions of fresh isooctane were added to elute the aliphatic fraction. The PAH fraction was eluted from the column with three 100-mℓ portions of benzene. The collected eluate can then be reduced in volume. The PAH were extracted next with dimethyl sulfoxide.

Recoveries from the Florisil® column were on the order of 90% or better, but depended strongly on the method of adsorbent pretreatment and on the type of sample studied. Relatively little loss of B(*a*)P from the column occurred during the isooctane elution. Some loss of B(*a*)P occurred during the benzene elution as a result of tailing. The problem was more severe with samples that had a low organic content or low B(*a*)P concentration. It was necessary to use batches of stationary phases prepared by the procedure described earlier to obtain consistent results. Fully activated Florisil® contains highly active acidic sites which are capable of leading to chemisorption of PAHs such as perylene, as was discussed in a paper by Snyder.[11] Such interaction leads to tailing and low recoveries. These sites can be effectively blocked by the addition of 1 to 2% water, and Dunn[10] has shown that this procedure led to excellent chromatographic reproducibilities.

Hurtubise et al.[12] has employed column chromatography to isolate the PAH fraction from other organics found in oil shale retort water. The dry column consisted of a 28.0-cm section of polyethylene tubing (0.25 in. I.D., Curtin Matheson Scientific, Inc.) which was loaded to a height of 23 cm with alumina (activity II to III, according to Brockman ICN Life Sciences Group). The packing was retained by a glass wool plug which was inserted on the exit end.

A 1.0-mℓ hexane concentrate of the sample was added to the column and developed with 5 mℓ of *n*-hexane-ether (19:1 v/v). The exact location of the PAH fraction must be ascertained in advance with standards. After the development was complete, the purple fluorescent band was located with a UV lamp and the column sliced to give a 5-cm section. The alumina from the sliced section was stirred for 30 min with 20 mℓ of 1,2-dichloroethane, then washed two more times with 10-mℓ dichloroethane portions. The combined extracts were then evaporated to dryness, and redissolved in hexane for further TLC separation.

III. THIN LAYER CHROMATOGRAPHIC ANALYSIS OF THE PAH FRACTION

A. Separation Studies

In addition to its effectiveness as a cleanup technique for isolation of the PAH fraction, TLC has often been used as the analytical technique for the qualitative and quantitative determination of individual PAH concentrations. The same adsorbents described earlier — silica, alumina, and acetylated cellulose, or combinations of these — are commonly employed as the stationary phase. The solvents mentioned earlier are also used as mobile phases.

Spotting procedures for TLC were discussed in Section II.B. Development procedures are more sophisticated for PAH separation and identification than for cleanup procedures. The spotted plate is dried either by a stream of air or by equilibration in an inert atmosphere. Glass development chambers are most often used, although specialized chambers with controlled temperatures and humidities are available. The simplest possible technique is multiple development with a single solvent system. The development is considered complete at the point at which a suitable resolution of PAHs has been effected. A variant of this method employs different solvent systems before each successive development. Multiple or stepwise development in two dimensions is also a method by which successful PAH separations can be effected. In this procedure, the sample to be analyzed is spotted in one corner of the plate and then allowed to develop in one dimension to a fixed distance. The plate is then removed from the developing chamber and dried. Upon rotation of the plate by 90°, the plate is developed with the same or different solvent system in the new direction. Resolution of PAHs with this technique is excellent.

Borneff[9] devised a two-dimensional TLC technique for separating the six WHO PAHs after an initial column chromatography cleanup. A mixed adsorbent stationary phase consisting of aluminum oxide and 40% acetylated cellulose in a 7:3 weight ratio was used. For the preparation of five TLC plates, 28 g of aluminum oxide was thoroughly mixed with 12 g of 40% acetylated cellulose and 65 g of ethanol. After the plates had been coated, they were activated for 30 min at 130° C in a drying oven. The sample extract was applied as one spot in one corner of the TLC plate about 1.5 cm from the two edges. The first development took place for 30 min with a mixture of *n*-hexane-benzene, (9:1 v/v). After drying and rotation of the plate by 90°, development proceeded for 60 min with a mixture of methanol-ether-water (4:4:1 v/v/v). Throughout the entire two-dimensional development, the TLC plate was kept in the dark. Since standards could not be spotted simultaneously with the sample, standard runs were carried out under identical conditions. The six WHO PAHs were clearly resolved under these analytical conditions.

Pierce and Katz[13] and Katz, Sakuma, and Ho[14] developed a two-stage TLC procedure for separating PAHs extracted from airborne particulate matter. Though most of the PAHs studied were of mol. wt. 276 and above (compounds unlikely to be found to a large extent in water), this technique was also applied to the benzopyrenes and benzofluoranthenes and is applicable to PAHs of any molecular weight.

The analytical scheme was pretested on a standard solution of PAHs, concentrations of approximately 0.1 mg/mℓ in toluene. In the first stage, the separation was carried out on a 20 × 20 cm TLC plate coated with neutral aluminum oxide. A 2.0-μℓ aliquot of standard solution was spotted 1.5 cm from the bottom of the plate, along with individual PAH standards. Pentane-ether (19:1 v/v) was the mobile phase. Development proceeded for 13 to 15 cm (20 to 30 min) in a sandwich chamber. This procedure provided a class separation of the 13-component PAH mixture into four groups. Each group was removed from the plate and the PAHs were eluted with 20 to 30 mℓ of hot

Table 3
RESOLUTION OF SEVERAL PAH BY TWO-STEP TLC TECHNIQUE

Compound	1st step: class separation aluminum oxide, pentane-ether (19:1 v/v) $R_{B(a)P}$[a]	2nd step: 40% acetylated cellulose, *n*-propanol-water-acetone (2:1:1 v/v/v) $R_{B(a)P}$[a]
Benzo(a)pyrene	1.00	1.00
Benzo(e)pyrene	0.98	6.61
Benzo(j)fluoranthene	0.99	1.51
Benzo(k)fluoranthene	1.00	3.07
Perylene	0.94	4.52
Benzo(ghi)perylene	0.89	6.78
Pyrene	1.18	6.40
Dibenz(a,h)anthracene	0.78	4.53

[a] $R_{B(a)P}$ relative R_F values with R_F of B(a)P equal to 1.00.

dichloromethane for 15 min. The extract was evaporated to dryness with dry nitrogen and was redissolved in toluene. Junk et al.[15] have pointed out that 10 to 80% of all but the extremely low vapor pressure solutes were lost using a free evaporation step of this sort. However, Katz studied only higher molecular weight PAHs and obtained recoveries ranging from 80 to 95%.

The PAHs in the four fractions were further resolved in the second stage of the TLC procedure. In this step, an 0.25-mm thick stationary phase of 40% acetylated cellulose was employed. Spotting and developing was carried out in the same manner as in the first stage. The mobile phase was *n*-propanol to acetone to water (2:1:1 v/v). Table 3 lists R_F values for the PAHs relative to B(*a*)P. It can be clearly seen that the benzopyrene and benzofluoranthene isomers were resolved. The general applicability of the procedure is limited by the evaporative step and the number of manual steps. If the volume reduction were to be performed with a micro Kuderna-Danish apparatus, the procedure should work well with PAHs of lower molecular weight. The procedure was tested using two-dimensional TLC with a mixed aluminum oxide-cellulose acetate stationary phase, but the inability to apply an internal standard to the same plate precluded its use for quantitative analysis. Qualitative analyses give excellent results with the two-dimensional scheme. In Borneff's work,[9] standards were applied to identical TLC plates and not to the same plate as the sample mixture. A comparison of relative deviations resulting from the use of external vs. internal standards is not available.

Mainwaring and McGuirk[16] have reviewed all of the basic TLC approaches for PAH analyses and have developed a simlified, two-dimensional TLC scheme based on the work of Pierce and Katz.[13] A mixed stationary phase, 20% acetylated cellulose to alumina (1:3) was activated at 100°C, and 50 $\mu\ell$-samples were developed. The development in the first direction was performed with a pentane to ether (19:1 v/v) mobile phase. The development in the orthogonal direction was carried out with ethanol to methylene chloride to water (20:10:1 v/v/v). The results indicated that the B(*a*)P moved to near the top of the plate after the first development. However, in the orthogonal second development, the B(*a*)P spot traveled behind all of the other spots on the chromatogram. Therefore, to take advantage of the high mobility of the B(*a*)P in the first direction and ensuing small relative shift in the second direction, Mainwaring and McGuirk developed a reversed two-dimensional technique, i.e., the second development

is in a 180° opposite direction from the first. The usefulness is apparent. The B(*a*)P spot which is near the top in the first development will be further resolved from the other spots in the second development as a result of lagging behind the other spots. Clearly, a third development could again be made in the opposite direction with the pentane to ether mobile phase. A major advantage of this approach is the ability to spot as many as 6 to 8 samples in a row on the bottom of the plate. Standards or multiple samples can be simultaneously run.

A number of other studies using a TLC separation step for PAHs have been published in addition to the work cited above. Bories[17] used polyamide as a stationary phase for the separation of a number of PAHs such as B(*a*)P, B(k)F, perylene, and coronene with a mixture of toluene and methanol (4:1 v/v) as the mobile phase. Shiraishi et al.[18] studied the separation of 28 PAHs on Kieselguhr G impregnated with 10% liquid paraffin with a 13:7 acetonitrile-water solvent mixture as the mobile phase. The separations obtained were poor compared to those obtained with the silica/alumina/acetylated cellulose substrate discussed earlier.

B. Methods of Detection

Qualitative analysis of the separated TLC spots can be easily effected by the use of a long wavelength UV lamp. PAHs can be identified by comparison of the R_F values to standards. Prior knowledge of the standard concentration even allows a visual semiquantitative analysis of unknown concentration through comparison of spot size. Borneff[9] has noted that reference chromatograms can be prepared which, if sprayed with a nonfluorescing preserving dispersion, are usable for a month or more as a semi-quantitative and qualitative visual standard if exposure to light is minimized.

Quantitative analyses of the PAHs are generally performed by one of two different methods: (1) measurement of the solution fluorescence after elution of the PAH spots from the TLC plate, or (2) measurement of the fluorescence intensity on the TLC plate. In the first method, the individual PAHs can be qualitatively identified on the thin layer plate, scraped off the plate, and eluted with an organic solvent. It is important that this step result in quantitative recovery. Automatic spot scrapers and elution devices are available which can process multiple samples with speed, accuracy, and precision. The solution of PAH is diluted to a specified volume, if necessary, and the fluorescence emission is measured at a predetermined emission wavelength. In some cases, the eluting solvent is unsatisfactory for follow-up fluorescence analysis due to a high level of a self-fluorescence. In such cases, the eluting solvent is usually evaporated with a stream of nitrogen, and the resultant residue is redissolved in a more suitable solvent. Care must be taken to guard against losses of volatile PAHs in the evaporation step. With the use of standards and prepared calibration curves, unknown concentrations can be determined.

In the second method, the fluorescence can be measured directly on the TLC plate. The excitation radiation is directed through slits onto the TLC plate, and the resulting fluorescence can be measured with a densitometer. The TLC plate is slowly moved past the slit at a constant rate and the fluorescence intensity is recorded on chart paper. The area of the resulting peak is proportional to fluorescence intensity which in turn is related to PAH concentration. Manual or electronic integration techniques can be used in the area measurements.

Katz et al.[14] and Pierce and Katz[13] determined the fluorescence intensities of PAHs separated by the TLC methods discussed earlier by eluting the spots from the plates with diethyl ether. These solutions were deoxygenated with a dry nitrogen stream and the solution fluorescences were measured. Quantitative results were obtained by comparison with standards. Scholz and Altmann[19] measured B(*a*)P in ground water by fluorescence analysis after prior TLC separation on silica gel. After locating and

Table 4
DETECTION LIMITS OF THE SIX WHO-REGULATED PAH WITH A TLC-FLUORESCENCE METHOD

Compound	Excitation wavelength (nm)	Emission wavelength (nm)	Absolute limit of detection (ng)	Limit in 60 ℓ of water (ng/ℓ)
Fluoranthene	365	458	140.0	2.3
Benzo(j)fluoranthene	365	427	7.5	0.1
Benzo(k)fluoranthene	365	428	5.0	0.1
Benzo(a)pyrene	365	427	10.0	0.2
Indeno(1,2,3-cd)pyrene	365	467	10.0	0.2
Benzo(ghi)perylene	365	416	20.0	0.3

scraping off the B(*a*)P spot from the plate, the B(*a*)P was eluted with cyclohexane. Upon evaporation of the cyclohexane to dryness and dissolution of the residue in dioxane, the B(*a*)P in solution was excited at 365 nm and the emission measured at 429 nm. A concentration of 0.1 ng/ℓ was detectable with relative errors of ± 15%.

Dunn[10] measured B(*a*)P in hexadecane solution fluorimetrically after removal of the PAHs from the TLC plate. A baseline was drawn between the minima in the fluorescence spectrum at 418 and 448 nm and the peak height at 430 nm was determined above this baseline. The sensitivity of the method was 0.1 μg/kg with a precision of 6%.

Basu and Saxena[20] have determined the fluorescence intensities of the six, WHO PAHs after prior TLC separation. Direct measurement of fluorescence on the plates at room temperature was performed with an Aminco® Bowman thin-film scanner attached to a spectrophotofluorimeter. The measurement was carried out in this way in order to avoid losses of PAHs during removal of the spots from the TLC plates and to eliminate errors from the introduction of stray fluorescence resulting from solvent impurities. Table 4 lists the excitation and emission wavelengths used for the six PAHs and the limits of detection. For all measurements, a quinine sulfate standard was used to correct for light source variations.

Woidich et al.[20a] were able to quantitatively estimate concentrations of PAHs after TLC separation by *in situ* fluorescence measurements. Plots of peak area vs. concentration of B(*a*)P in picograms were linear from 50 to 10,000 pg, and the limit of detection was 10 pg. The fluorescence measuring conditions for 14 other PAHs were listed. Kaschari and Reiter[21] measured the fluorescence intensities of B(*a*)P, B(k)F, IP, B(b)F, and perylene in oil distillates and refinery waste-waters, after TLC separation on acetylated cellulose. They were able to detect between 0.1 and 2 ng of each PAH.

Hurtubise et al.[12] measured the fluorescence intensity of B(*a*)P directly on a TLC plate. A Kontes® densitometer was used and the recorded peak heights, corresponding to the fluorescence intensity of the standards, were plotted vs. the amount of B(*a*)P in nanograms. Unknown concentrations of B(*a*)P could then be directly determined from the calibration curve. The limit of detection was found to be 0.9 ppb. With larger sample sizes spotted onto the TLC plate, this limit was lowered to 0.08 ppb.

C. Future Trends

The overwhelming advantages of a TLC technique for the analysis of PAHs arise from the simplicity of the operation and the inexpensive experimental requirements. A quick screening of the PAH fraction can be performed to determine whether followup GC, HPLC, or other techniques are necessary. The separation efficiency of TLC is high for isomeric PAHs. Fluorescence detection of the resolved spots allows subnanogram levels

of PAHs to be determined. It is our conclusion that a backup TLC analysis system should be considered by all investigators.

IV. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

A. Introduction

The application of HPLC to the analyses of PAHs in environmental samples has increased significantly in the last few years. Two factors are primarily responsible for the utilization of HPLC in environmental analytical laboratories. The first factor is the development of chemically bonded stationary phases for HPLC which have greatly improved the separation of PAH isomers. For example, chrysene/benz(a)anthracene (B(a)A)/triphenylene are baseline resolved with a C_{18} column packing. The highly successful separations achieved with this packing are reflected by the fact that all the papers which reported on the use of HPLC at the Fourth International Symposium on PAHs in Columbus, Ohio, October 1979, utilized C_{18} stationary phases in the separation system.[22] Although performance variations exist among the C_{18} packings available from various manufacturers, it is clear from personal discussion at the Symposium that the performance of C_{18} packed columns for PAH analyses is unsurpassed at present.

The second factor is the development of highly sensitive fluorescence detectors which have extended the detection limits of PAHs to picogram and even subpicogram levels. A useful discrimination is provided by this type of detector since almost all PAHs are capable of fluorescing, whereas many other organic pollutants are nonfluorescing. With the use of a fluorescence detector system, both an excitation and an emission wavelength can be selected, and this provides another degree of selectivity. A critical evaluation of these and several other developments in PAH analyses are presented.

B. Separation Studies

1. Introduction

HPLC can be viewed as a logical extension of classical column chromatography. An essential difference between column chromatography and HPLC is the size of the column packing materials. In column chromatography, the particle size is large enough to permit the mobile phase to flow by gravity alone; in HPLC, the microparticulate packing material (5 to 10 μm) requires the use of a high pressure pumping and injection system. The high surface to volume ratio of the microparticulate packing results in an HPLC separation efficiency comparable to that obtained by GC.

The efficiency of HPLC separations is influenced by a number of factors: column dimension, sample volume, pressure, mobile phase flow rate, and the composition of the stationary and mobile phases. This review will consider specifically the selection of the column packing and an appropriate mobile phase system best suited to that packing for the separation of PAHs.

Historically, the first types of packings investigated were the traditional column chromatography materials, e.g., silica and alumina. For these polar adsorbents, nonpolar mobile phases such as hexane, heptane, pentane, benzene, toluene, and chloroform were employed. Separation efficiencies of PAHs with these systems were only fair. The degree of separation was determined by selective adsorption on the silica or alumina surface. Attempts to improve the separation by coating the silica or alumina with various substances had limited success because of difficulties in achieving homogeneous surface coverage. Column bleed presented additional problems. The primary requirements of the mobile phase for the silica and alumina packings were the nonpolarity and the "dryness" of the solvent. A number of n-alkanes or mixture of n-alkanes with benzene, toluene, or diethyl ether have been used.

With the development of chemically bonded stationary phases, many of the problems of the traditional chromatographic systems are reduced. Stationary phase bleed is minimized with the chemical bonding of the stationary phase to the support. Furthermore, the nature of the silica surface is altered by the bonded nonpolar groups such as the octadecyl group, so that the need for scrupulously dry solvents to prevent the deactivation of the silica surface no longer exists. The technique utilizing a nonpolar stationary phase and a polar mobile phase is called "reversed phase" HPLC, as opposed to normal phase HPLC with polar adsorbent packings. The mechanism of separation of PAHs in reversed phase HPLC mainly involves the differential solubility of the PAHs in the mobile phase, although interactions with the C_{18} group or unreacted support surface may also affect the retention mechanism.

Since the mechanism of separation in HPLC depends on the differential solubilities of the PAHs in the mobile phase, the proper choice of solvents is critical. Generally, the PAHs are eluted with a mixed solvent system rather than isocratically, i.e., with a single solvent. The term "gradient elution" is applied when the composition of the mixed solvent is changed linearly from a water-rich to an organic-rich mixture during the course of the run.

Snyder et al.[23] estimate that nearly 70% of all HPLC separations employ gradient elution. A similar solution exists in the field of gas chromatography where temperature programming predominates over isothermal GC separations. The following advantages for gradient elution separations employing a linear solvent strength program are:[23] (1) regular band spacing throughout the chromatogram, (2) constant band widths in the chromatogram, for all bands, (3) comparable resolution or effective plate number for early and late eluting bands, and (4) conceptual simplicity which permits the prediction of separations with variation of experimental parameters. Optimum conditions for a gradient elution program depend on a knowledge of several instrumental parameters. The organic solvent to be used with water depends on the compounds to be separated; acetonitrile and methanol are the most commonly used for PAHs. The compositions generally reported range from 20 to 50% organic solvent to 100% organic solvent. The gradient steepness, i.e., the percentage increase in organic solvent depends upon the particular solvent and the column dead time. For a 1-min dead time with acetonitrile, a 6.7% gradient is optimal for maximum resolution. An excellent discussion on the "fine-tuning" aspects involved in the design of a solvent program can be found in Dolan et al.[24]

2. Reversed Phase High Performance Liquid Chromatographic Separations of PAHs — C_{18} Bonded Phases

Grushka and Kitka[25] recently reviewed the use of chemically bonded stationary phases in HPLC. Although most of the reported HPLC studies have been carried out with commercially available C_{18} bonded phases, it is informative to understand how the bonded phases are prepared. At present, silica gel is the most commonly employed support surface. This surface has a fixed number of hydroxyl groups which can be reacted with mono-, di-, or trichlorooctadecylsilanes (Figure 3). The first step involves heating the silica surface under vacuum to 150°C to remove bonded water. To prepare the C_{18} bonded stationary phase, trichlorooctadecylsilane is reacted with the silica gel (Figure 3c). For reactions with dry solvents, a monomeric (one C_{18} group) stationary phase should result. If there are traces of protic impurities in the solvent, the free chloro group can be hydrolyzed with the resultant hydroxyl group capable of further reaction. This leads to a polymeric stationary phase, as seen in Figure 3d. The free hydroxyl groups left after the bonding of the C_{18} group can be removed by silanization. The extent of polymeric coating can have an effect on separations.[26] The extent of surface coverage can be calculated with details found in Reference 25.

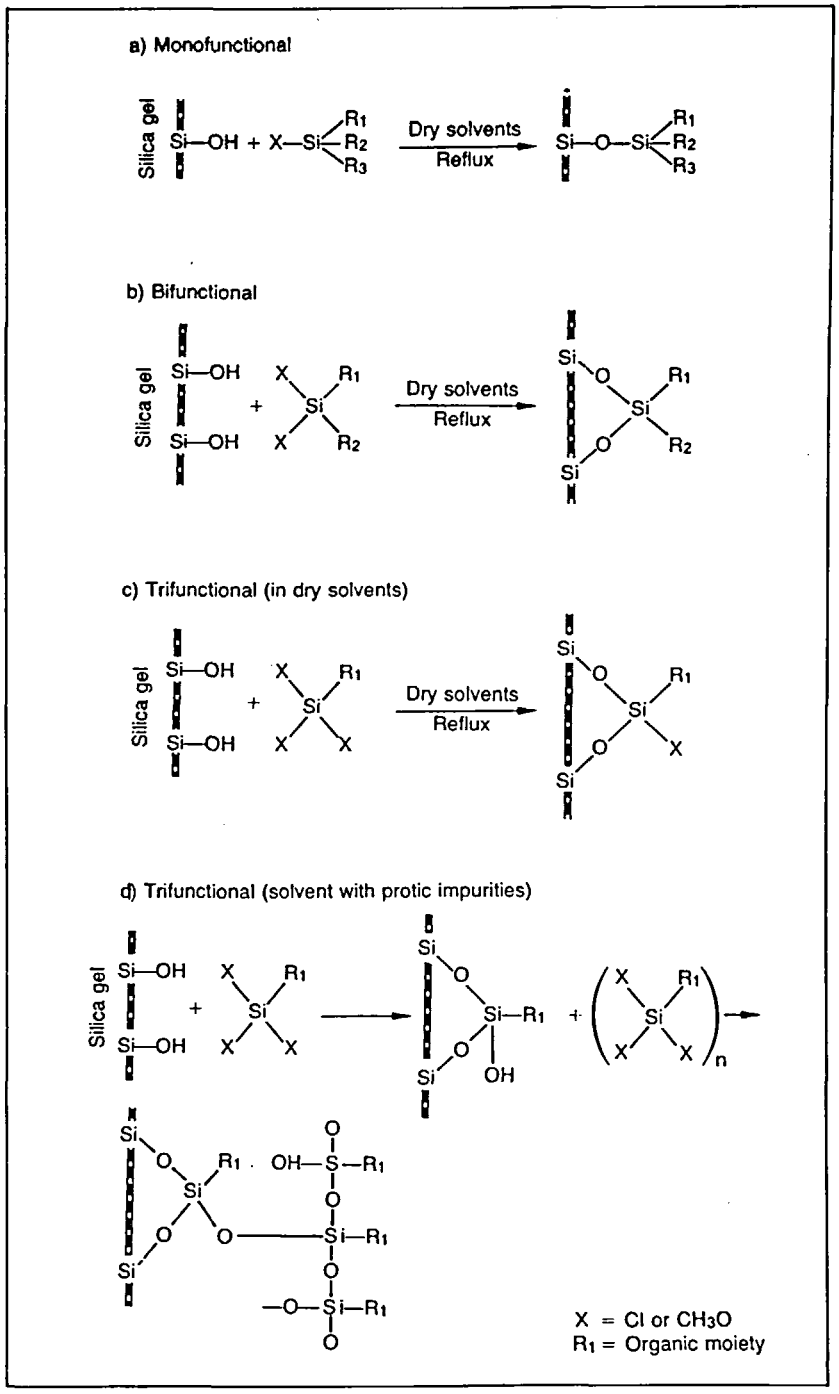


FIGURE 3. Schematic of reaction of chloro- or alkoxysilanes and silica gel.

Wise et al.[26] recently compared the performance of several commercially available C_{18} columns. The column packings were: LiChrosorb® RP-18, Partisil-5® ODS, Dupont® Zorbax® ODS, Micropak® CH-10, Nucleosil® 10 C_{18} (Vydac® 201 TP, essentially identical to Perkin Elmer® HC-ODS), Radial Pak® A, and Micropak® MCH-10. Studies were

Table 5
CHROMATOGRAPHIC CONDITIONS

Liquid chromatograph	Perkin-Elmer® Series 3, with dynamic stirrer accessory, Rheodyne® Model 7120 injection valve, 10 μL loop
Column	Perkin-Elmer® HC-ODS, 0.26 × 25 cm, 10 μm, C_{18} packing, Part No. 089-0716
Mobile phase	Acetonitrile in water T1: 50—50%, 15 min T2: 50—100%, 8 min, linear T3: 100—100%, 27 min T Purge: 20%, 5 min T Equil: 50%, 15 min 0.5 mℓ/min flow rate room temperature
Detector	Perkin-Elmer® Model 650-10LC, spectrofluorimeter; bandpass, 12 nm
Data collection	Perkin-Elmer® Sigma 10 chromatography data station

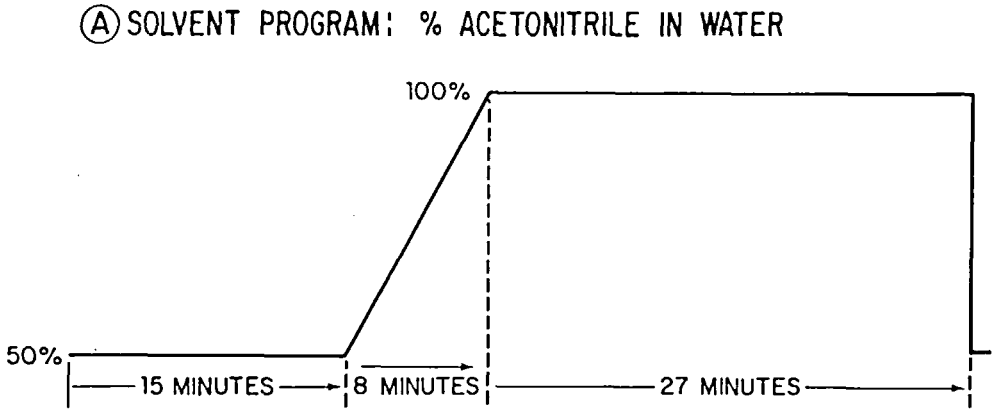


FIGURE 4. Solvent program for chromatographic analysis showing the pump control profile.

performed on the separation of PAH standards using a mobile phase of acetonitrile and water with a gradient ranging from 70 to 100% acetonitrile over 40 min. They found that only the Vydac® column gave a baseline separation of chrysene and B(*a*)A. Only two column packings were considered satisfactory; the Dupont® Zorbax ODS had the highest efficiency, i.e., gave the sharpest peaks, and the Vydac 201 TP® exhibited the best selectivity towards PAHs. The polymeric Zorbax® column is useful for complex or "dirty" samples where a high efficiency is advantageous, and the Vydac® column is useful for the resolution of individual PAHs.

The work being conducted at Perkin-Elmer by Ogan et al.[27-29] is at the forefront of the studies on PAH separations. Extracts of various aqueous samples have been analyzed by HPLC coupled with fluorescence detection. The extracts were obtained by liquid-liquid extraction of 1-ℓ samples with dichloromethane, followed by Kuderna-Danish concentration. A model solution of 16 PAHs, including the 15 on the EPA Priority Pollutant list, was also studied.

The chromatographic conditions for the analysis are shown in Table 5. The solvent program depicted in Figure 4 was employed. Equilibration of the column between runs was carried out by passing a 20% acetonitrile mobile phase through the column for 6 min.

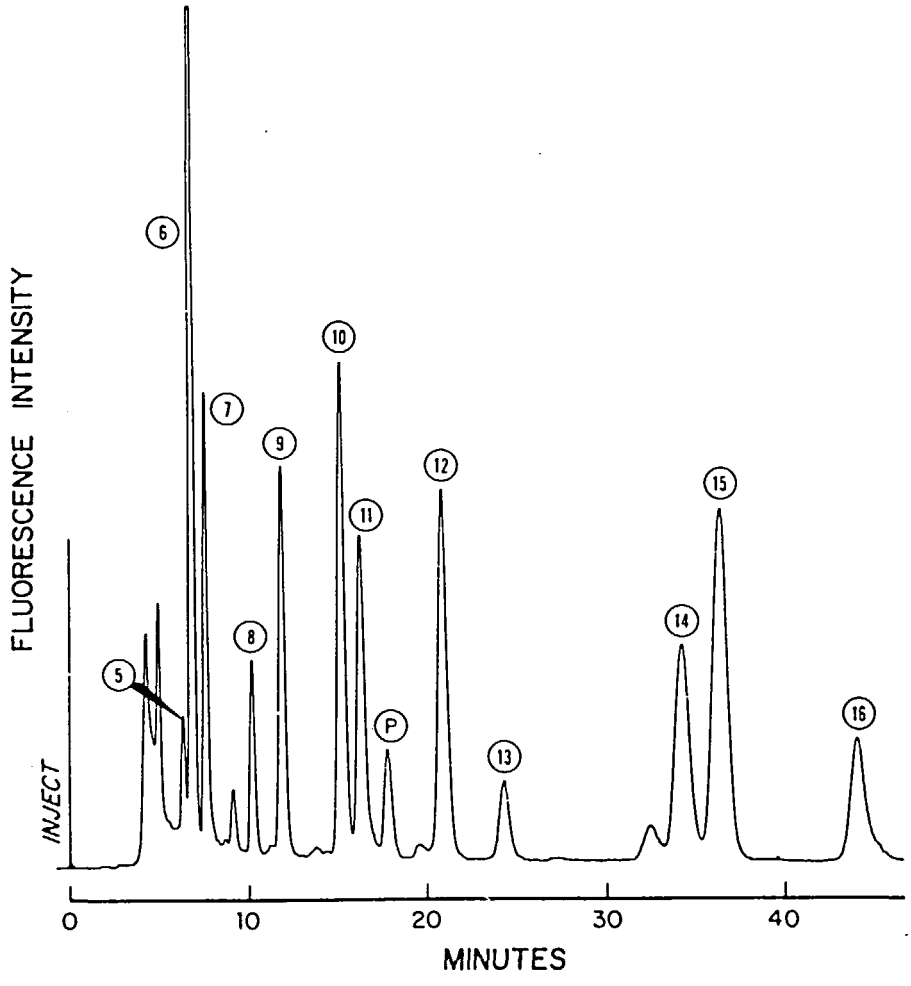


FIGURE 5. Chromatogram of 16 PAH standards using isocratic elution (80% acetonitrile in water at a flow rate of 0.5 mℓ/min) at room temperature. Peak legend: (1) naphthalene, (2) acenaphthene, (3) fluorene, (4) phenanthrene, (5) anthracene, (6) fluoranthene, (7) pyrene, (8) benz(a)anthracene, (9) chrysene, (10) benzo(e)pyrene, (11) benzo(b)fluoranthene, (P) perylene, (12) benzo(k)fluoranthene, (13) benzo(a)pyrene, (14) dibenz(a,h)anthracene, (15) benzo(ghi) perylene, (16) indeno(1,2,3-cd)pyrene.

This was followed by 50% acetonitrile for 15 min. The sample loop was rinsed with 30 $\mu\ell$ of acetonitrile and air dried before the introduction of a 15 $\mu\ell$ sample of extract into the loop.

Figure 5 illustrates an example of the PAH separation by HPLC that they obtained with the use of isocratic elution (80% acetonitrile in water) at room temperature. It can be seen that anthracene and phenanthrene are only partially resolved, and peaks 14 to 16 are beginning to broaden significantly. Figure 6 illustrates the separation of the 16 PAHs achieved by Ogan's group with the solvent program of Figure 4. Dibenz(a,h) anthracene (D(a,h)A) and B(ghi)P were only partially resolved. Complete baseline resolution was achieved for all of the other isomers that are generally difficult to separate: phenanthrene/anthracene, B(a)A/chrysene/triphenylene, and B(e)P/benzo(b)fluoranthene/B(k)F/B(a)P. Peak shapes were sharp all the way to and including IP, molecular weight 278, which eluted after 40 min.

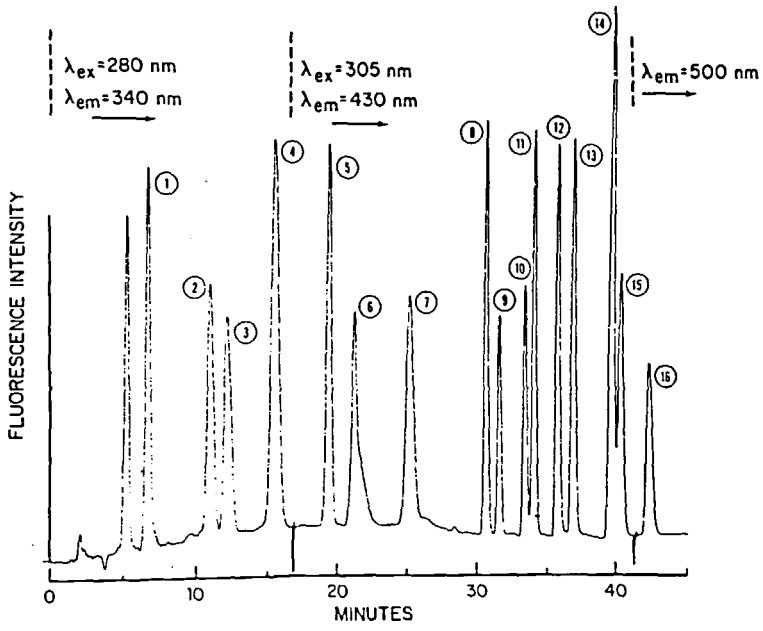


FIGURE 6. Chromatogram of 16 PAH standards using the solvent program of Figure 4, and conditions of Table 5. For peak identification, see legend in Figure 5. The tail on peak 6 is a solvent impurity.

Ogan et al.[27] have studied the separation characteristics of individual PAHs as a function of mobile phase composition, and they found that acenaphthene and fluorene were best resolved with a reduced mobile phase strength. Mobile phase strength increases with the percentage of organic solvent in the mixture, reaching a maximum at 100% organic solvent. D(a,h)A and B(ghi)P were best resolved at concentrations of acetonitrile near 100%. Relative selectivity factors, α, were calculated according to the equations:

$$\alpha = \frac{k'_i}{k'_r} \quad (1)$$

$$K' = \frac{V_i - V_o}{V_o} \quad (2)$$

where k'_i is the capacity ratio of the particular PAH, k'_r is the value for B(a)P, V_i is the retention volume of the solute, and V_o is the dead volume. Figure 7 illustrates the change in α as the percentage of acetonitrile increases.

Retention times were strongly dependent on the length of time allowed for equilibration of the apparatus. Retention times during a single day were reproducible to 0.8 to 1.6% for PAHs up to fluoranthene and 0.5 to 0.7% for PAHs of higher molecular weight. Day-to-day precision was slightly less.

Results for real environmental samples which were analyzed by the same procedure are shown in Figures 8 and 9. Figure 8 illustrates the analysis of an extract of fly ash wash water and Figure 9 an extract of a river water sample. The quantitative detection procedures used by Ogan are described in Section IV.C.

Das and Thomas[30] have employed an HPLC method using a 25 cm × 0.21 cm column

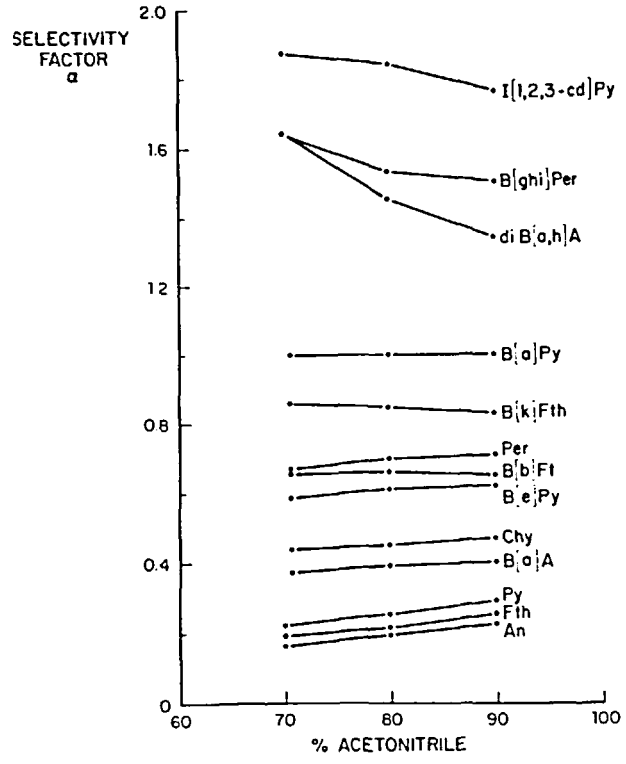


FIGURE 7. Selectivity factor, α, relative to benzo(a)pyrene, as a function of acetonitrile content of the mobile phase (isocratic elution).

picked with a Dupont Zorbax® ODS C_{18} stationary phase. An isocratic elution procedure was employed with a solvent mixture of 82% acetonitrile in water as the mobile phase to analyze for PAHs in samples of coke oven waste water and extracts from particulate emissions. The isomers B(a)A/chrysene and B(k)F/B(e)P/perylene were unresolved.

Some of these same difficulties were encountered by Nielsen[3] in HPLC analyses of gasoline and crankcase oil samples containing PAHs. The PAH content of the samples were isolated from non-PAH fractions by means of an initial TLC separation on silica gel, details of which were given earlier in Section II.B. For the HPLC separation, a Zorbax® ODS column, 25 cm × 0.46 cm, was employed as the analytical column with a Nucleosil® 5 C_{18} precolumn, 6 cm × 0.46 cm. After prolonged use of the analytical column, the column capacity was noted to decrease; therefore, the short precolumn served a protective role. Upon incorporation of the Nucleosil® precolumn, 600 analyses resulted in no decrease in capacity. An isocratic elution mode was used with a mobile phase of methanol to water (8:1).

Nielsen[3] has tabulated relative retention times for several PAHs employing the above-mentioned HPLC conditions (Table 6). He found that the B(a)A/chrysene/triphenylene isomers were totally unresolved, as were the 1- and 9-methylanthracene isomers. There seemed to be a reasonable separation of the other components of the important PAH fraction: MW-252, B(e)P, B(*a*)P, B(k)F, and perylene, as well as an excellent separation of the two dibenzanthracene isomers.

Sorrell et al.[31] have studied the possibility of PAH contamination in drinking water from distribution pipes coated with corrosion-inhibiting coal tar and petroleum-based

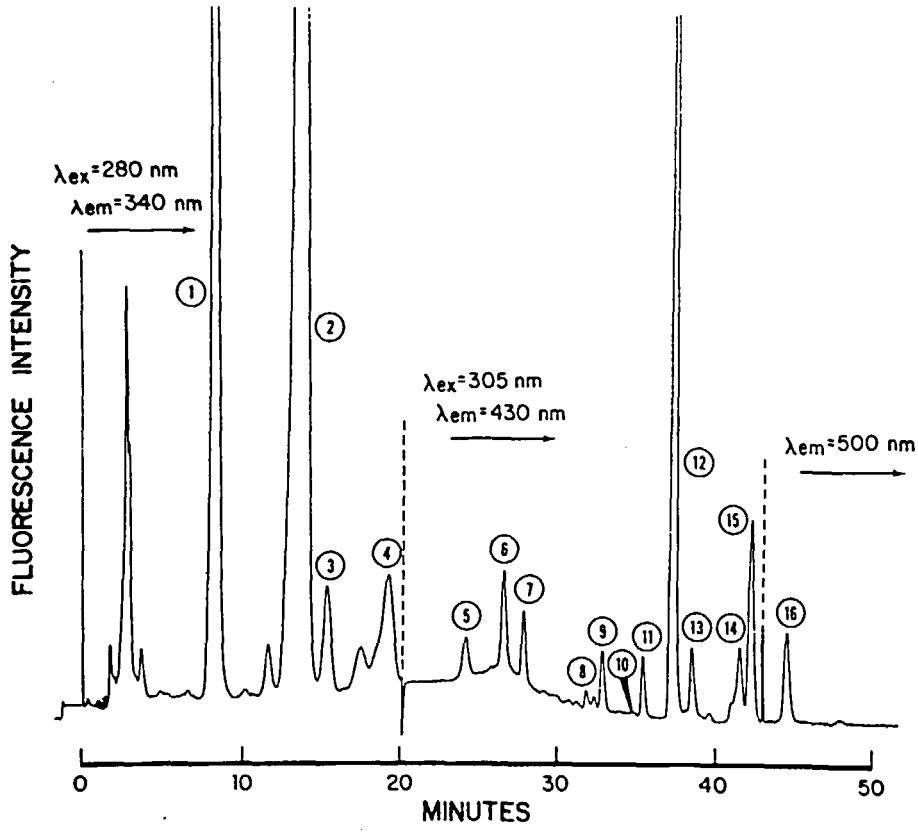


FIGURE 8. Chromatogram of the extract of fly ash wash water obtained with the hplc conditions given in Table 5. For peak identification, see Figure 5.

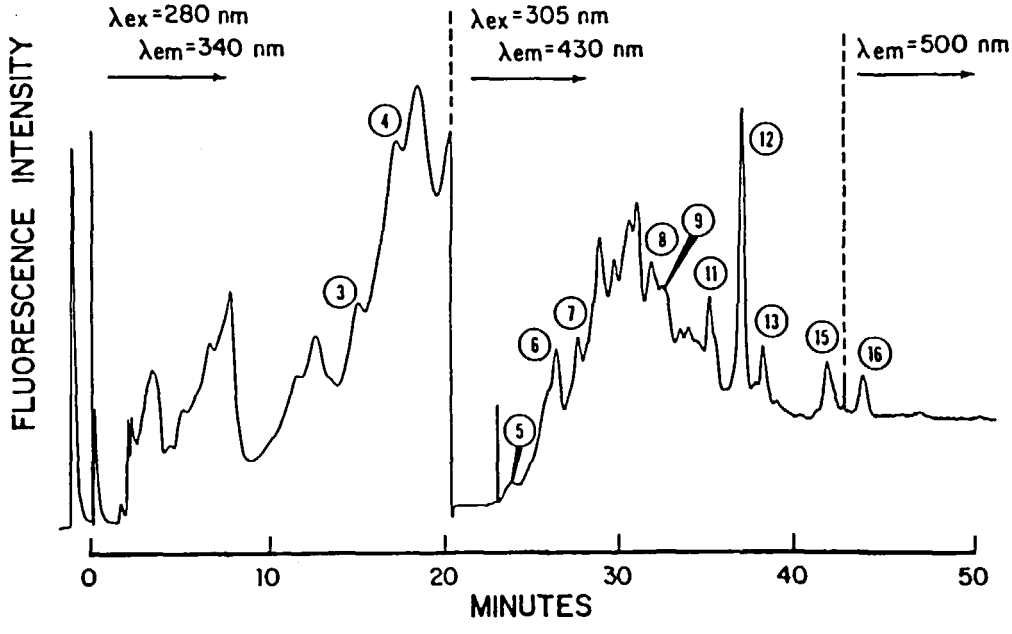


FIGURE 9. Chromatogram of the extract of a river water sample obtained with the HPLC conditions in Table 5. For peak identification, see Figure 5.

Table 6
RETENTION TIME DATA FOR PAH

Compound	Relative retention time[a]
Phenanthrene	0.93
Anthracene	1.00
Fluoranthene	1.20
1-Methylanthracene	1.31
9-Methylanthracene	1.34
Pyrene	1.35
2-Methylanthracene	1.42
2,3-Benzofluorene	1.69
Benz(a)anthracene	1.75
Chrysene	1.76
Triphenylene	1.76
Benzo(ghi)fluoranthene	1.78
9,10-Dimethylanthracene	1.83
Benzo(j)fluoranthene	2.2
Benzo(e)pyrene	2.4
Perylene	2.5
Benzo(k)fluoranthene	2.6
Benzo(a)pyrene	2.9
Dibenz(a,c)anthracene	3.1
Dibenz(a,h)anthracene	3.6
Benzo(ghi)perylene	4.3
Indeno(1,2,3-cd)pyrene	4.6
Coronene	5.7

Note: Pre-column, Nucleosil® 5 C_{18} 6 cm × 0.46 cm; main column, Zorbax® ODS 25 cm × 0.46 cm; solvent, methanol-water (8:1) at 1.0 mℓ/min; pressure, 1700 psi; temperature, 21°C.

[a] Anthracene approximately 11.5 min.

sealants. The water held in a test pipe system was subjected to liquid-liquid extraction with cyclohexane and the extract was concentrated in a Kuderna-Danish apparatus. The PAH fraction was separated on alumina prior to the HPLC analysis with a Waters, μBondapak C_{18} column. Isocratic elution at a flow rate of 1 mℓ/min with a 70% acetonitrile mobile phase was used.

The cleanup procedure resulted in three fractions of PAHs eluted from the alumina column. Despite this initial fractionation, B(a)A and chrysene as well as B(b)F and B(k)F were only partially resolved in the HPLC step. However, the cleanup did result in the separation of perylene and B(*a*)P from each other as well as from the benzofluoranthenes. In addition, B(ghi)P, IP, and D(a,h)A could all be identified without interference.

Fox and Staley[32] used a 25 cm × 0.8 cm stainless steel column packed with Dupont Zorbax® ODS for the analysis of PAHs found in airborne particles. The PAHs were eluted isocratically with a 7:3 (v/v) methanol to water mobile phase. In this investigation, B(a)A and chrysene could not be completely resolved, although they could be distinguished by use of selective modulation techniques (see Section IV.C). B(*a*)P and B(e)P were resolved in test samples (Figure 10), but in environmental samples B(e)P and B(k)F were unresolved. In a chromatogram of a sample of airborne particulate matter (Figure 11), the identified PAHs are numbered, but other unidentified components may mask additional PAHs present in smaller concentrations.

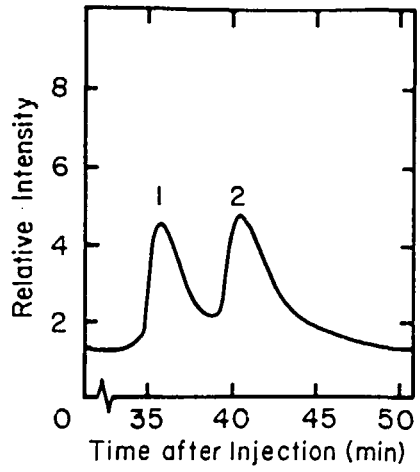


FIGURE 10. Hplc/fluorescence trace of a mixture of benzo(e)pyrene (1) and benzo(a)-pyrene (2). Chromatographic conditions: 25 cm × 0.80 cm I.D. Zorbax® ODS column; mobile phase, 13:7 (v/v) methanol-water, 65°C, 1400 psi; flow rate, 1.5 mL/min.

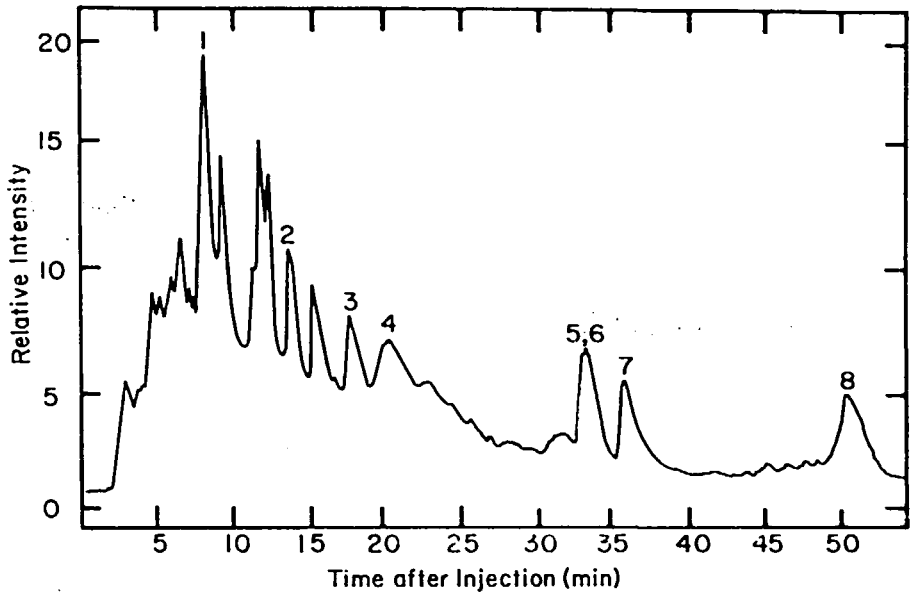


FIGURE 11. Partial hplc/fluorescence trace of a benzene extract of an atmospheric particulate matter sample collected in the Baltimore Harbor Tunnel. Chromatographic conditions: 25 cm × 0.80 cm I.D. Zorbax® ODS column, 7:3 (v/v) methanol-water, 65°C, 1600 psi; flow rate, 1.7 mL/min. Peak legend: (1) fluoranthene, (2) pyrene, (3) benz(a)anthracene, (4) chrysene, (5) benzo(e)pyrene, (6) benzo(k)fluoranthene, (7) benzo(a)pyrene, (8) benzo(ghi)perylene.

Dong et al.[33] also investigated the problem of the analysis of PAHs in airborne matter using HPLC conditions identical to those of Fox et al.[12] The poor resolution of isomeric PAHs that they obtained with isocratic elution strongly suggests the advisability of gradient elution for this class of separation.

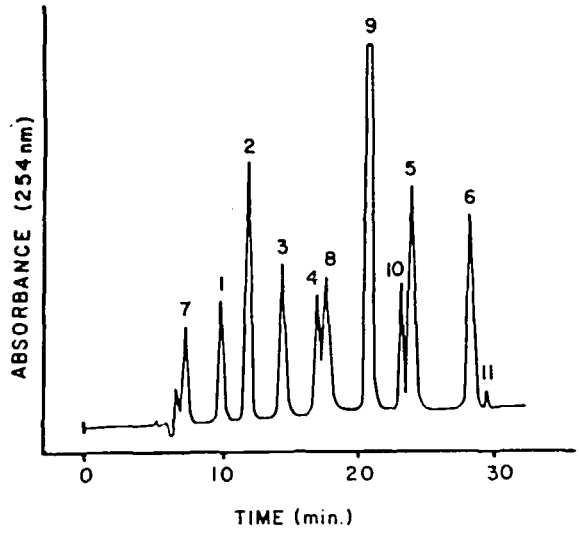


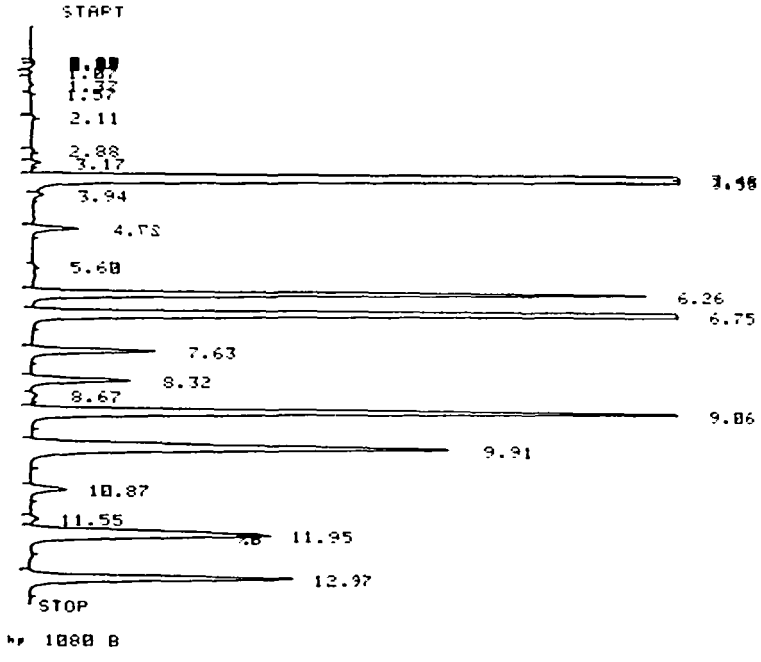
FIGURE 12. Separation of an 11 PAH standard. Chromatographic conditions: column, Partisil® 10-ODS; gradient, linear from 60/40 methanol-water (v/v) to 100% methanol in 34 min; column pressure, 630 psi; temperature, ambient. Peak legend: (1) benzene, (2) naphthalene, (3) biphenyl, (4) phenanthrene, (5) fluoranthene, (6) pyrene, (7) chrysene, (8) perylene, (9) benzo(a)pyrene, (10) dibenz(a,h)anthracene, (11) coronene.

Krstulovic et al.[34,35] have studied PAHs in various air samples from the Rhode Island area, with a Partisil® 10-ODS column, 25 × 0.46 cm. Gradient elution was used with an initial mobile phase of 60/40 (v/v) methanol-water. Over a 34-min period, the methanol concentration was linearly increased to 100%, which amounts to a rise of 1.2%/min. Fairly good separations were achieved on a model solution of eleven PAHs, ranging from naphthalene to coronene (Figure 12); however, the isomeric pairs B(*a*)P/perylene and fluoranthene/pyrene were not 100% resolved. Although there is a dependence on the column dead time and flow rate, the 1.2% rate of increase in solvent strength used by these investigators is quite low compared to the optimum value of 6.7% recommended by Dolan et al.[24] It appears that either the full advantages of gradient elution were not realized or that the μ-Bondapak® column was not extremely selective towards PAHs.

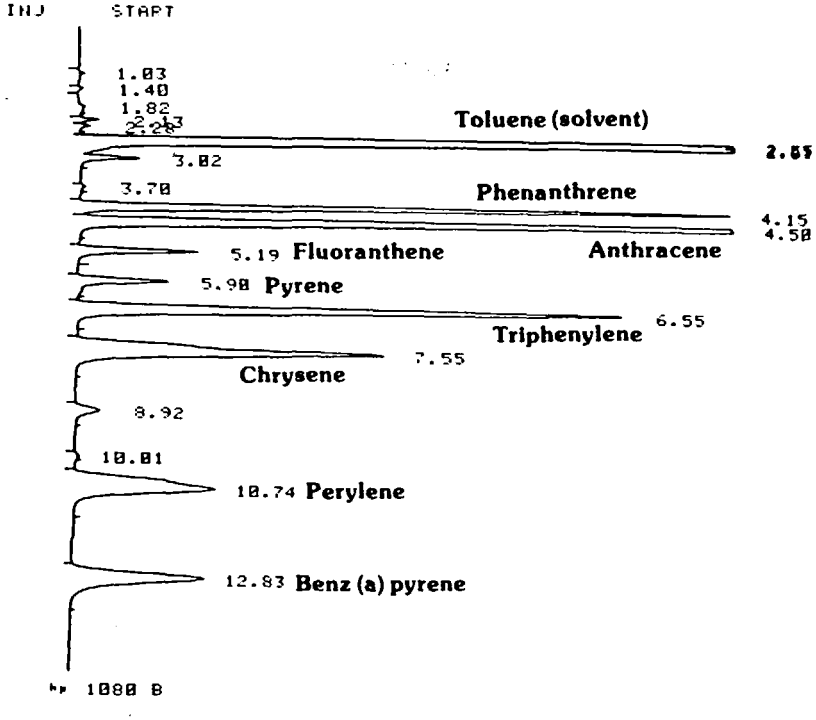
Euston and Baker[36] have studied the separation of eight PAHs by linear gradient and isocratic elution procedures with a LiChrosorb® RP-18 column. Although a 12-min run with a linear gradient from 60 to 95% acetonitrile in water provided a much better column efficiency than isocratic elution at 82.5% acetonitrile, they preferred the isocratic mode of separation because solvent mixing during the gradient run introduced noise. Both runs are illustrated in Figure 13. It can be seen from this figure that B(e)P co-eluted with perylene, and B(a)A with chrysene in both cases.

Kasiske et al.[37] have studied separations of the six WHO PAHs by HPLC on Nucleosil® C_{18} with a mobile phase of 100% methanol. There was poor separation of the benzofluoranthenes, although B(*a*)P was resolved, as were the other three PAHs. Samples from river water and waste dump seepage were analyzed.

Chmielowiec and Sawatzky[38] have performed an interesting study on the effect of temperature on HPLC separations of PAHs. They used a 31.4 cm × 0.46 cm column packed with Chromegabond-C_{18}® (ES Industries, Marlton, N.J.) and used acetonitrile to water (30:20 v/v) as the mobile phase. Figure 14 shows a separation of PAHs carried out



A



B

FIGURE 13. (A) Separation of an eight PAH standard by gradient elution technique on a C_{18} reversed phase packing; gradient, 70% acetonitrile in water (v/v) to 95% acetonitrile in water in 12 min. (B) Isocratic separation of the same PAHs, mobile phase, 82.5% acetonitrile in water.

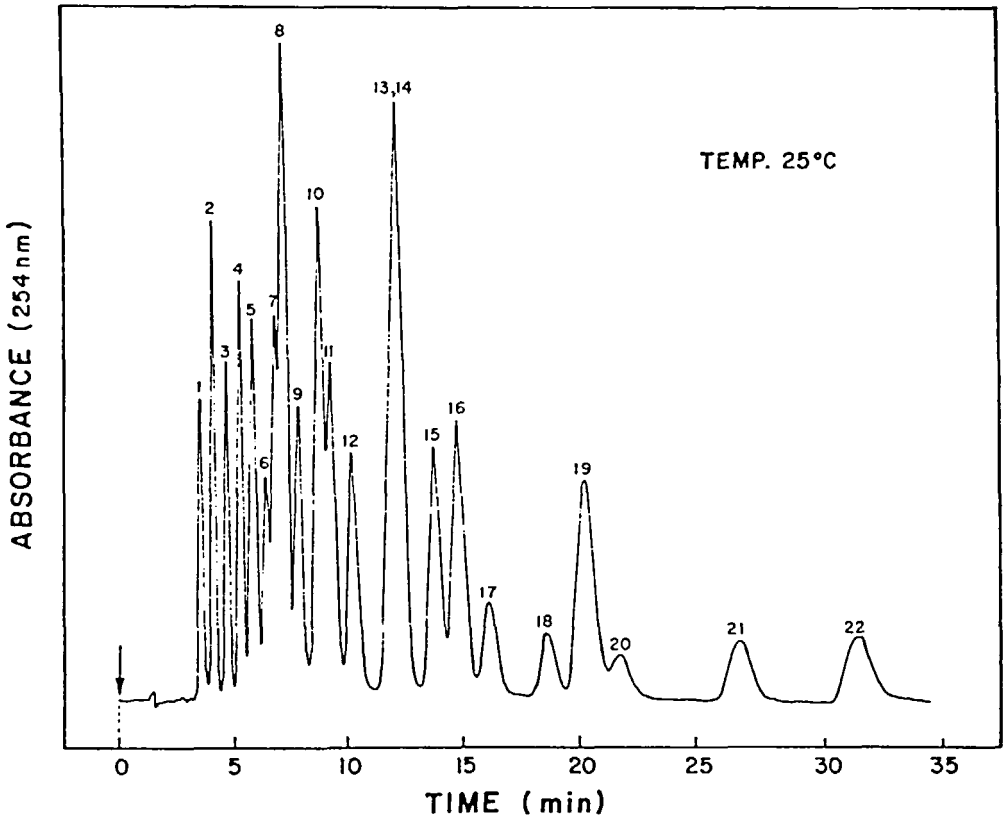


FIGURE 14. Isothermal separation of PAHs on silica-C_{18} column with mobile phase of acetonitrile-water (4:1, v/v). Peak legend: (1) naphthalene, (2) biphenyl, (3) fluorene, (4) phenanthrene, (5) anthracene, (6) 2-phenylnaphthalene, (7) fluoranthene, (8) 9-methylanthracene, (9) pyrene, (10) triphenylene, (11) difluorenyl, (12) chrysene, (13) 1,3,5-triphenylbenzene, (14) 2,2'-dinaphthyl, (15) 2-phenylanthracene, (16) benzo(b)fluoranthene, (17) benzo(k)fluoranthene, (18) benzo(a)pyrene, (19) 9,10-diphenylanthracene, (20) dibenz(a,h)anthracene, (21) benzo(ghi)perylene, (22) 1,2,3,4-dibenzopyrene.

under isothermal conditions at 25° C, and Figure 15 depicts the same HPLC separation with a linear temperature gradient of 2.6° C/min up to 50° C. Compounds 13 and 14, which were unresolved under isothermal conditions, were resolved when temperature programming was employed. The peak widths were sharpened and the analysis time was reduced. The thermodynamic basis for temperature effects on separations was outlined by Chmielowiec and Sawatzky[38] and involves the consideration of both entropy and enthalpy factors. Their initial studies were the basis for their conclusion that controlled temperature variation has the potential for providing significant improvement in resolution for compounds that are difficult to separate, e.g., PAHs. In addition, the elution of higher molecular weight PAHs can be accelerated. They also suggested that instrumentation and mobile phase parameters require further study.

Lankmayr and Muller[4] have studied the separation of PAHs by HPLC with a LiChrosorb® RP-18 stationary phase packing and an acetonitrile-water (85:15 v/v) mobile phase. The k' values obtained for the PAHs studied are shown in Table 7. The separations at MW:228, MW:252, and MW:278 should be especially noted. Although chrysene and B(a)A were essentially unresolved, these two compounds were resolved from triphenylene. A partial resolution of B(b)F, perylene, B(e)P, B(k)F, and B(*a*)P was achieved, and B(*a*)P was well separated from the rest. The two dibenzanthracene isomers were completely resolved and the components of the MW:278 fraction, IP, and

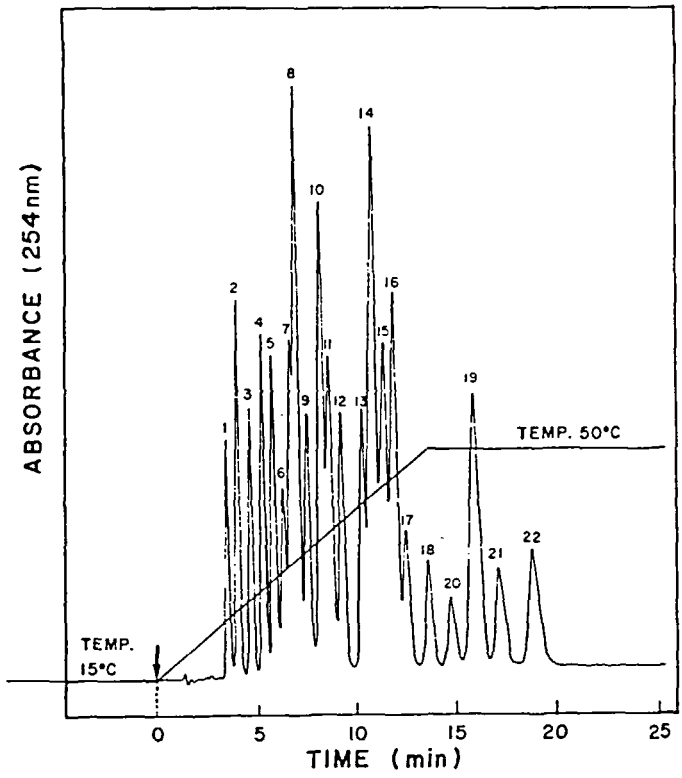


FIGURE 15. Gradient temperature separation of compounds shown in Figure 14.

Table 7
k' VALUES OF SEVERAL PAH ON LiCHROSORB® RP-18

Compound	k'
Fluoranthene	2.09
Pyrene	2.71
Triphenylene	2.82
11H-Benzo(a)fluorene	2.88
Benz(a)anthracene	3.29
Chrysene	3.35
Benzo(b)fluoranthene	4.89
Perylene	4.99
Benzo(e)pyrene	5.05
Benzo(k)fluoranthene	5.33
Dibenz(a,c)anthracene	5.84
Benzo(a)pyrene	6.17
Dibenz(a,h)anthracene	7.01
3-Methylcholanthrene	9.21
Indeno(1,2,3-cd)pyrene	9.29
Benzo(ghi)perylene	9.55
Coronene	18.96

Note: LiChrosorb® RP-18 (5 μm), 150 × 3.2 mm, mobile phase: acetonitrile-water (85:15).

Table 8
RELATIVE RETENTION TIMES FOR PAH ON PPS

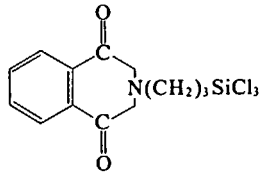
Compound	Relative retention time
Fluoranthene	0.48
Benz(a)anthracene	0.55
9,10-Dimethylanthracene	0.61
12-Methylbenz(a)anthracene	0.64
7-Methylbenz(a)anthracene	0.73
Benzo(ghi)fluoranthene	0.75
Benzo(b)fluoranthene	0.82
Benzo(k)fluoranthene	0.82
Dibenz(a,h)anthracene	0.93
Benzo(a)pyrene	1.00
Benzo(e)pyrene	1.07
3-Methylcholanthrene	1.07
Perylene	1.20
Indeno(1,2,3-cd)pyrene	1.45
Benzo(ghi)perylene	1.86
Anthanthrene	1.86

Note: Conditions: mobile phase, methanol-water (90:10) at a flow rate of 1.5 mℓ/min. Retention time of benzo(a) pyrene was 11.5 min.

B(ghi)P were partially distinguishable. No results were presented on the reproducibility of k′ values.

3. Alternative Reversed Phase Systems

Phthalimidopropylsilane (PPS) developed by Hunt et al.[39] is the only successful alternative reversed phase packing for HPLC separations of PAHs that has been employed both in a reversed phase and normal mode for PAH separations. The structure of the starting material which is bonded to silica is shown below:



The support for this packing material is Partisil® 5, a microparticulate silica gel (5 μm), which is refluxed with 2 *M* HCl for 2 hr and then sequentially rinsed with water and methanol until neutral. After overnight drying at 100° C, 7 g of the treated silica gel is reacted in a dry flask with 25 mℓ of dry xylene and 10 mℓ of 3-phthalimidopropyltrichlorosilane for 2 hr. At the end of the reflux period, 2 mℓ of methyltrichlorosilane are added and refluxing is continued for 30 min to deactivate the silica surface as completely as possible. Upon cooling, the PPS is washed thoroughly with xylene to remove unreacted silanes and is then washed with methanol.

This packing was used in a 25 cm × 0.46 cm stainless steel column with a mobile phase of methanol to water (90:10). The retention time results of an HPLC run on a PAH mixture are shown in Table 8. Chrysene was not one of the included compounds so that its separation from B(a)A cannot be evaluated. However, B(k)F, B(e)P, B(*a*)P, and

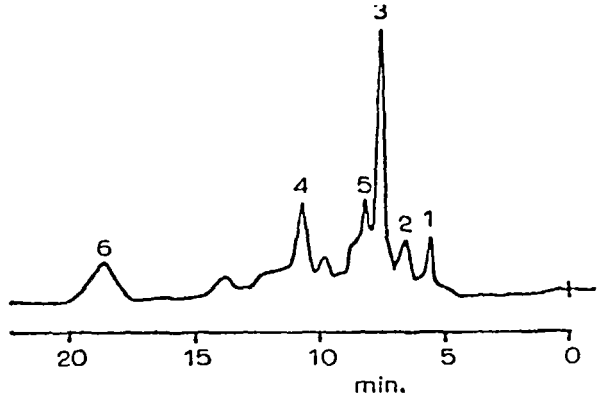


FIGURE 16. Separation of the six World Health Organization PAHs on a PPS packed column. Chromatographic conditions: mobile phase, 10% toluene in hexane; flow rate, 2 mL/min. Peak legend: (1) fluoranthene, (2) benzo(k)fluoranthene, (3) benzo(b)fluoranthene, (4) benzo(a)pyrene, (5) indeno-(1,2,3-cd)pyrene, (6) benzo(ghi)perylene.

perylene were completely separated. The chromatogram of an extract from mussels is shown in Figure 16. It would be interesting to see whether the application of a proper gradient elution procedure could further improve PAH resolutions. The detection of B(ghi)P in only 20 min is noteworthy.

4. Normal Phase PAH Separations

Hunt et al.[40] employed PPS as a stationary phase in a normal mode separation of PAHs by HPLC with a mobile phase of toluene/hexane (1:10). The six WHO PAHs were studied. Complete baseline separation of the six PAHs — fluoranthene, B(k)F, B(b)F, B(*a*)P, IP, and B(ghi)P — was accomplished.

Crathorne and Fielding[41] examined the separation of the six WHO PAHs using a Partisil®-5 packing, microparticulate silica gel. Although the benzofluoranthene isomers were only 50% resolved using a mobile phase of 0.01% acetone in hexane, resolution of all six was accomplished (Figure 17). They noted that small changes in the mobile phase composition could dramatically affect separations; therefore, care is essential in preparing the mobile phase and in activating the column. The most successful approach involved a rigorous exclusion of water from the solvents used in the mobile phase.

A number of other bonded phase packing materials have been devised for PAH separation studies. Lankmayr and Muller[4] studied silica gel supports modified with NH_2 and NO_2 groups in normal mode systems. Among these, LiChrosorb® NH_2 (10 μm) and Nucleosil® 5 NO_2 are commercially available. Mobile phases were selected on the basis of good resolution and time of analysis. For LiChrosorb® NH_2, isooctane dried over alumina was chosen as the mobile phase while isooctane containing 10% dichloromethane was used for separations with Nucleosil® NO_2. Some k' values for the PAHs separated are listed in Table 9. Fluoranthene and pyrene were unresolved on Nucleosil® NO_2, although there was a partial separation of the components comprising the MW:228 and MW:252 fractions.

Lochmuller and Amoss[42] developed a bonded charge transfer type of stationary phase packing for the separation of alkyl-substituted PAHs. Vespalec[43] has investigated the

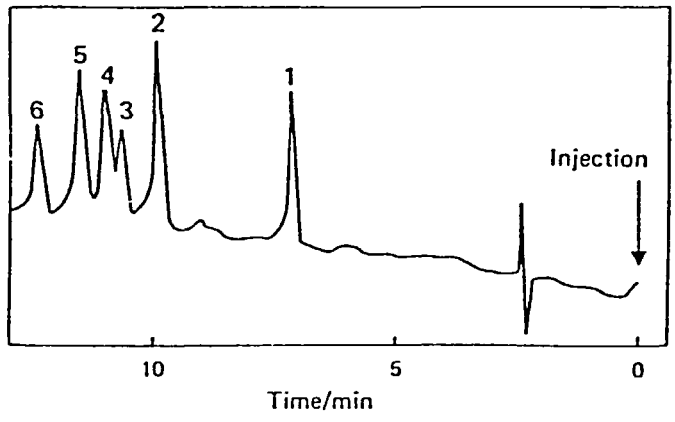


FIGURE 17. Separation of the six World Health Organization PAHs. Chromatographic conditions: column, 25 cm of Partisil-5®, mobile phase, 0.01% acetone in hexane; flow rate, 1.5 mL/min; UV detection, 287 nm. Peak legend: (1) fluoranthene, (2) benzo(a)pyrene, (3) benzo(k)fluoranthene, (4) benzo(b)fluoranthene, (5) benzo(ghi)perylene, (6) indeno(1,2,3-cd)pyrene.

Table 9
k′ VALUES OF SEVERAL PAH ON LiCHROSORB® NH_2 AND NUCLEOSIL® NO_2

Compound	k′ Nucleosil® NO_2	Compound	k′ LiChrosorb® NH_2
Fluoranthene	1.45	Pyrene	1.18
Pyrene	1.47	Fluoranthene	1.48
Benz(a)anthracene	1.94	Triphenylene	1.86
3-Methylcholanthrene	2.09	Chrysene	2.00
Chrysene	2.16	Perylene	2.07
Triphenylene	2.33	Benz(a)anthracene	2.12
Benzo(k)fluoranthene	2.44	3-Methylcholanthrene	2.32
Benzo(a)pyrene	2.79	Benzo(a)pyrene	2.57
Benzo(b)fluoranthene	3.05	Benzo(b)fluoranthene	2.72
Benzo(e)pyrene	3.19	Benzo(e)pyrene	2.72
Perylene	3.37	Benzo(k)fluoranthene	2.78
Dibenz(a,h)anthracene	3.64	Indeno(1,2,3-cd)pyrene	3.38
Indeno(1,2,3-cd)pyrene	3.87	Benzo(ghi)perylene	3.52
Benzo(ghi)perylene	4.32	Dibenz(a,c)anthracene	3.55
Dibenz(a,c)anthracene	4.52	Dibenz(a,h)anthracene	3.67
Coronene	6.45	Coronene	4.60

Note: Nucleosil® 5 NO_2, 150 × 3.2 mm, mobile phase: isooctane dichloromethane (9:1); LiChrosorb® NH_2 (10 μm), 250 × 3.2 mm, mobile phase: isooctane dried over alumina.

selectivity of 1,2,3-tris(2-cyanoethoxy) propane coated as a liquid on silica gel for the separation of PAHs. The separations of isomeric PAHs were poor in both cases.

Popl et al.[44,45] have studied the effect of the molecular structures of various PAHs on their adsorptivity on silica gel and alumina using a normal phase system with dry n-pentane as the mobile phase. Adsorption energies of PAHs were calculated from measured retention volumes. Retention indices were calculated in a manner analogous to

the Kovats' index system for GC. The studies with alumina and silica gel focused primarily on the relationship indices to factors such as type of ring system, alkyl branching, and carbon number. Popl et al.[46] have extended this form of systematic study towards the chromatography of aromatic hydrocarbons on macroporous polystyrene gel.

Wise et al.[47] at the National Bureau of Standards have carried out studies which will enable predictions to be made regarding separations on the C_{18} phases, which are the most commonly employed for PAH separations. They have devised a two-stage analytical scheme for analyses of PAHs in complex environmental samples such as crude oil fractions. In the first step, a polar chemically bonded stationary phase, μBondapak® NH_2 (an aminopropylsilane bonded chemically to microporous silica, 10 μm) was used. Polar compounds were separated on this material. When a nonpolar mobile phase such as hexane was used with this column, a hydrocarbon class fractionation was achieved. Saturated hydrocarbons eluted before the aromatic hydrocarbons, and a linear relationship existed between the elution volume and the number of aromatic rings. With this separation procedure, PAH fractions, based on the number of rings, were obtained. The logarithms of the retention indices have been calculated in the same manner as Popl et al.[44,45] and are presented in Table 10.

The retention of a particular PAH on μ Bondapak® NH_2 was affected only minimally by alkyl side chains. This is in contrast to separations on silica or alumina where the retention increases upon addition of alkyl groups. The reason for this behavior is explained in terms of the retention mechanism. In normal phase HPLC, retention times are governed solely by the interaction between the π electrons of the PAHs and the π electron system of the polar amino group; therefore, little increase in retention is expected.

The second stage of Wise's work involved the separation on μ Bondapak® C_{18}. A linear gradient mobile phase program was employed, ranging from 50 to 100% acetonitrile in water. The gradient was spanned in 30 min. Wise's group reported an excellent resolution of a number of alkyl homologues; however, the isomeric PAHs such as B(a)A and chrysene and phenanthrene and anthracene were not well resolved. Retention indices for the analysis on the C_{18} column are listed in Table 10.

C. Methods of Detection

The two most common methods of detection are based on the strong UV absorption by PAHs and their characteristically high fluorescence intensities. The fluorescence method is more sensitive and selective towards PAHs. Advantages of the fluorescence method are as follows: (1) the fluorescence technique allows the selection of two characteristic wavelengths, one for excitation and one for emission; (2) for strongly fluorescing substances such as PAHs, the fluorimetric method is more sensitive to lower concentration levels than UV methods; (3) since not all compounds fluoresce, all potentially UV-absorbing nonfluorescing interferences are not detected. The sensitivity advantage of fluorescence detection over UV detection is illustrated in Figure 18.

The simplest approach for detection of the PAH eluants from HPLC is to use a UV detector set at 254 nm. If the column is capable of completely resolving the PAHs, the detector will give a signal for every compound. However, since no column is able to afford complete resolution, interference effects will always exist.

The efficiency of UV detection can be improved by selectively monitoring more than one wavelength; however, a prior knowledge of the components of the sample is necessary in order to preselect the optimum UV absorption wavelength of each PAH. Krstulovic et al.[34] used six different wavelengths to analyze a mixture of 10 PAHs. Figure 19 illustrates the relative sensitivity of various PAHs at different wavelengths.

Table 10
RETENTION INDICES FOR AROMATIC HYDROCARBONS ON TWO-STAGE μBONDAPAK® NH_2 — μBONDAPAK® C_{18} SYSTEM

Compound	Logarithm of the retention index (log I)	
	μBondapak® NH_2[a]	μBondapak® C_{18}[b]
Two-ring aromatics		
Naphthalene	2.00	2.00
2-Methylnaphthalene	1.96	2.59
2,3-Dimethylnaphthalene	2.08	3.47
2,6-Dimethylnaphthalene	1.94	3.15
1,5-Dimethylnaphthalene	1.93	3.00
2,3,6-Trimethylnaphthalene	2.00	3.54
1,4,6,7-Tetramethylnaphthalene	2.05	3.86
2-Ethylnaphthalene	1.92	3.14
Biphenyl	2.25	2.57
3-Ethylbiphenyl	2.20	3.46
Acenaphthene	2.00	2.73
Three-ring aromatics		
Fluorene	2.61	2.78
Dibenzothiophene	2.75	2.98
Anthracene	2.95	3.02
2-Methylanthracene	2.92	3.63
9,10-Dimethylanthracene	2.95	3.82
Phenanthrene	3.00	3.00
1-Methylphenanthrene	2.98	3.43
p-Terphenyl	3.40	3.94
Four-ring aromatics		
Fluoranthene	3.39	3.42
Benzo(a)fluorene	3.46	3.74
Benzo(b)fluorene	3.53	3.78
Pyrene	3.68	3.51
Naphthacene	3.93	—
Benz(a)anthrancene	4.00	4.00
Triphenylene	4.00	3.82
Chrysene	4.03	3.94
Five-ring and larger aromatics		
Benzo(a)pyrene	4.30	4.57
Perylene	4.47	4.43
Benzo(ghi)perylene	4.61	>5
Indeno(1,2,3-cd)pyrene	4.72	>5
Dibenz(a,c)anthracene	4.93	4.84
Dibenz(a,h)anthracene	4.93	>5
Picene	>5	4.93
Benzo(b)chrysene	5.00	5.00

[a] Hexane mobile phase (pentane produces identical chromatographic results but flow reproducibility is poorer).
[b] Acetonitrile-water mobile phase.

The main advantage of UV detectors is that they are simple and easy to operate. In practice, their usefulness for PAH detection of HPLC eluants is generally confined to a subordinate role. UV detectors are often used in series with fluorescence detectors as a means of providing a general response before the fluorescence detector provides a more selective response.

There are a number of commercially available fluorescence detectors; however, all of

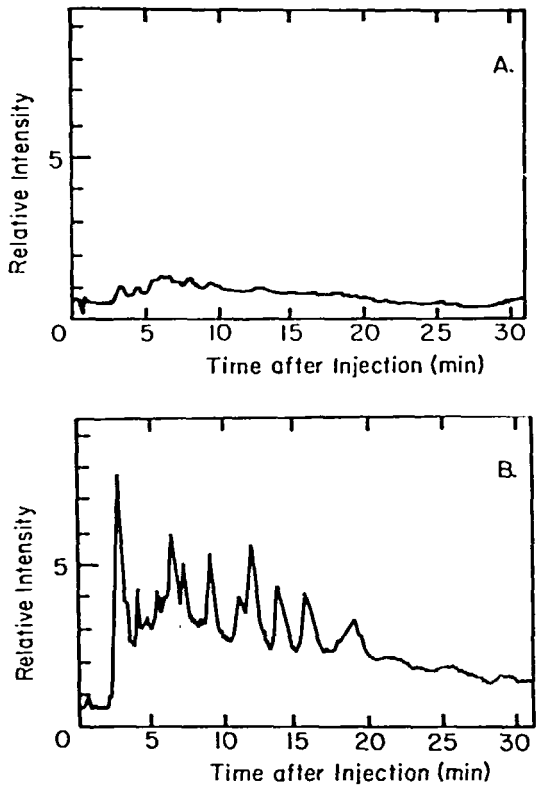


FIGURE 18. Partial HPLC/UV absorption (A) and fluorescence (B) traces of a benzene extract of atmospheric particulate matter collected in the Baltimore Harbor Tunnel. These traces were determined simultaneously. Chromatographic conditions: 25 cm × 0.8 cm I.D. Zorbax® ODS column, 13:7 (v/v) methanol-water, 65°C, 1400 psi; flow rate, 1.5 mL/min.

them can be classified into two major types. The first, which is the simplest and least expensive, is a modified filter fluorimeter which allows excitation and emission at set wavelengths. A set of interchangeable filters allows a reasonable choice of operating wavelengths. A more sophisticated detector can be built around a spectrofluorimeter with its variable wavelength monochromator. Any emission wavelength is then available, best detected with a high through-put, cut-off filter.

A typical optical arrangement for fluorescence detection devised by Slavin et al.[48] is shown in Figure 20. The optical system included a xenon capillary flash lamp light source, an interchangeable filter for choosing excitation wavelengths, and a continuously variable interference filter for choosing an emission wavelength. The spectrum bandpass could be limited to roughly 14 nm. The xenon lamp provided high light intensities from 260 to 656 nm. Emission wavelengths of 390 to 750 nm could be selected with the variable interference filter. A reference photomultiplier served as a monitor to correct for any intensity variations in the wandering of the xenon lamp. A rectangular quartz flow cell was used since this geometrical shape was claimed to produce less stray light than a circular flow cell.[48]

Two general methods of determining the fluorescence intensities of the individual PAHs are available. The most common method is the dynamic method in which the column effluent passes through a flow cell, typically 5 to 50 $\mu\ell$ in volume. However, a

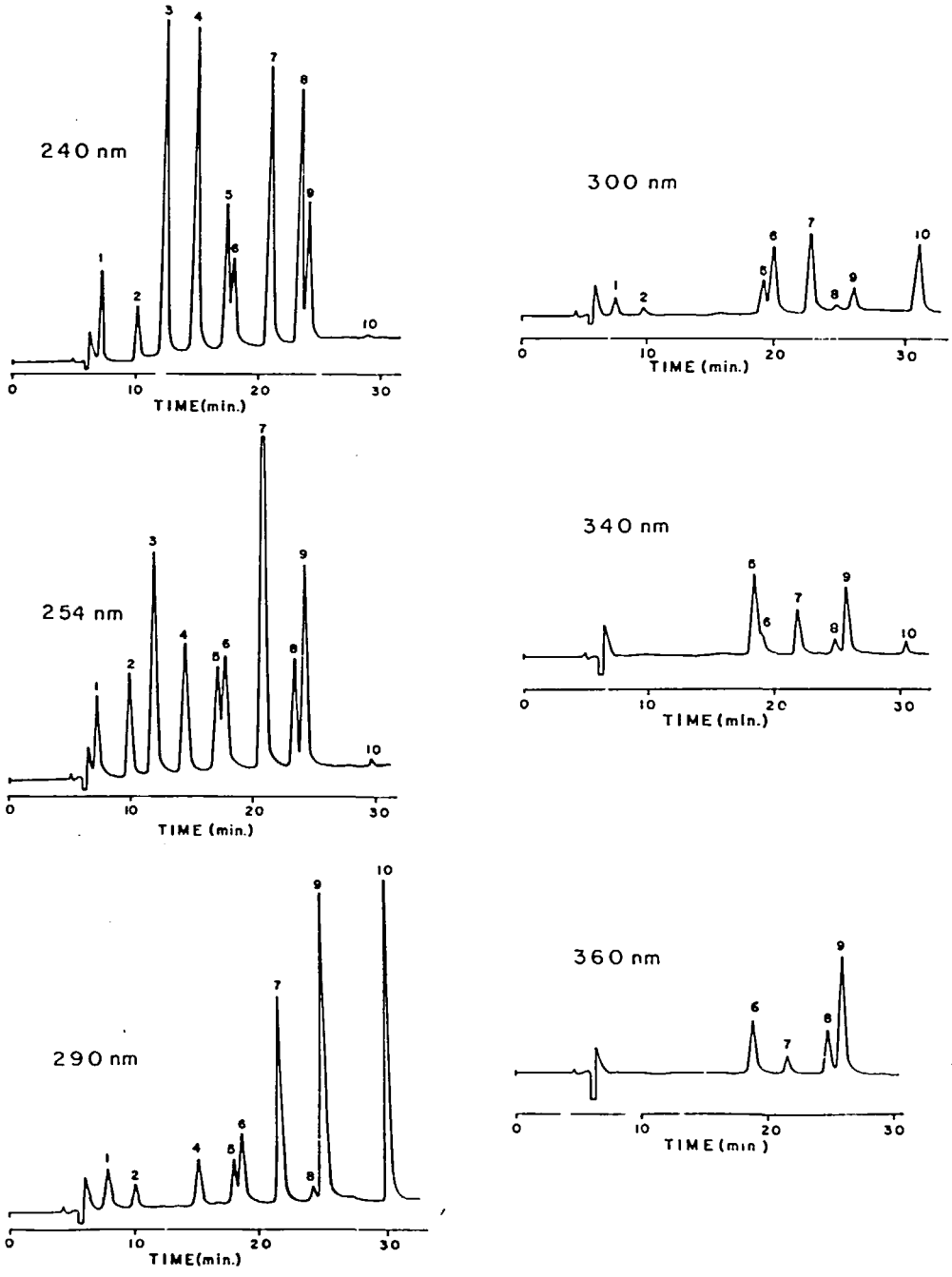


FIGURE 19. Detection of PAHs at different wavelengths, order of elution: (1) benzene, 37.9 μg; (2) naphthalene, 1.55 μg; (3) biphenyl, 1.50 μg; (4) phenanthrene, 0.65 μg; (5) fluoranthene, 1.00 μg; (6) pyrene, 0.65 μg: (7) chrysene, 0.65 μg; (8) perylene, 1.00 μg; (9) benzo(a)pyrene, 0.35 μg; (10) coronene, 0.91 μg.

static method is also used; in this method, the fluorescence spectra are obtained by stopping the chromatographic flow and measuring the fluorescence signal.

When all of the operating parameters have been established, a calibration curve is set up to determine the working range of concentration. A standard should be available for

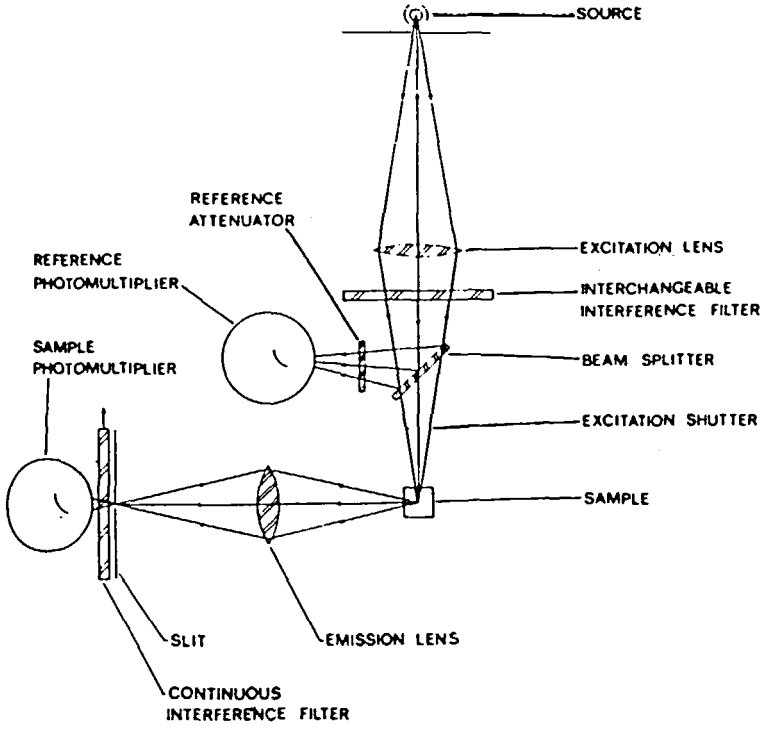


FIGURE 20. Typical optical arrangement of an HPLC fluorescence detector.

each PAH present. The need for calibration is especially important in fluorescence detection since the total fluorescence intensity depends on a number of variables: (1) the filter quality, (2) the purity of the mobile phase, (3) the quantum yield or fluorescence efficiency, (4) the solute fluorescence lifetime, and (5) potential quenching effects by other dissolved species such as oxygen. Self-absorption or inner quenching of the fluorescing species can affect the detector response at high concentrations, but this is generally not a problem at the low concentrations of PAHs found in water, even after preconcentration steps.

The problem of fluorescence quenching by dissolved oxygen has not been widely studied. Fox and Staley[32] have noted that dissolved oxygen in solution can quench the excited singlets of the PAHs. A fourfold increase in the fluorescence intensity of B(*a*)P was observed when the mobile phase was completely deoxygenated. The difficulty in reproducing the deoxygenation of the mobile phase may be the explanation of the small number of published studies in this area.

It is necessary to compare the detection limits and sensitivities of PAHs on the basis of available HPLC detectors and the inherent problems in UV and fluorescence detection. Christensen and May[49] of the National Bureau of Standards have compared detection limits of PAHs for fixed wavelength UV, variable wavelength, filter fluorimeter, and spectrofluorimetric detectors. In order to present meaningful results, they address the problem of defining sensitivity and detection limit. It was noted that sensitivity is most often expressed in terms of detection limits, which are then defined in different manners in the literature. One convention is to define the detection limit as "the smallest possible amount of material which gives rise to an observable peak". Detection limits based on this interpretation depend on the type of chromatography employed as well as on the actual instrument and detector. To determine the sensitivity for a specific detector, one

Table 11
COMPARISON OF METHODS FOR DETERMINING DETECTION LIMITS

Criterion for determining limit	Naphthalene	Phenanthrene	Pyrene	Chrysene	Benzo(a)pyrene
2 x Range	0.16	0.031	0.10	0.056	0.039
3 x Standard deviation	0.12	0.024	0.07	0.042	0.029
3 x RMS area	0.19	0.021	0.085	0.042	0.025

Note: UV absorbance detector at 254 nm; detection limit in ng injected.

must know the actual concentration of material present in the detector sample cell which gives rise to an observable peak.

Ambiguities of the definition may result because an "observable peak" can be defined in a number of ways. The usual criterion is that a peak is observed if the signal to noise ratio is greater than or equal to two.[49] Christensen and May[49] noted that even the definition of noise is subject to variation. They suggest that in the case of random noise, twice the noise range might be as many as six times the standard deviation about the mean. As a result, the probability that a peak is real is greater than 99.9%, a criterion the authors consider too stringent. The noise level can also be characterized by the standard deviation of the signal about the mean or baseline. A real peak with a probability of 99.7% is then considered to be any peak greater than three times the standard deviation. Another definition of noise level is commonly used with electronic integrators, where the noise level is defined as the root mean square average of the peak areas produced by the noise excursions. Any peak three times greater in area than this average can be considered a true peak. The probability is also 99.7% in this case. For each of these definitions, the limits of detection for PAHs using a 254-nm UV detector are compared in Table 11.

Christensen and May[49] have applied their definitions of detection limits to studies of the efficiencies of several HPLC detectors for the determination of PAHs. Limits of detection for a fixed-wavelength (254 nm) UV detector, three variable-wavelength UV detectors, three filter fluorimeters, and one spectrofluorimeter, are presented in Tables 12 and 13. The detection limits were calculated in terms of the amount of PAH injected into the chromatograph and, alternatively, the concentration of PAH in the detector cell. Several commercially available detectors denoted by A, B, C, etc. were used by NBS in these studies. Picogram quantities of PAHs were observed with all of the detectors. Christensen and May concluded that the optimum detector system consists of a 254 nm fixed UV-wavelength detector in series with a spectrofluorimeter. The UV detector gives a general response to all PAHs, and the spectrofluorimeter can be used for the quantitative determination of individual PAHs.

Ogan et al.[27] used a Perkin-Elmer® Model 650-10LC fluorescence detector for the determination of PAHs separated by the HPLC method discussed earlier. The excitation and emission spectra of authentic samples of the 16 PAHs studied were recorded. The important emission and excitation peaks of the 16 PAHs are listed in Table 14. The overlapping of the spectra of some PAHs necessitates the selection of compromise wavelengths for several PAHs. Three groups of PAHs, each with distinct excitation and emission wavelengths, were set up (Table 15). These groups were distinguishable on the basis of retention times as well as on the basis of the change in selected excitation and emission wavelengths between phenanthrene and anthracene. An enhanced fluorescence intensity of IP was realized by shifting the emission wavelength to 500 nm. The addition of standard PAHs to sample extracts served as a means of positive identification of

Table 12
DETECTION LIMITS IN TERMS OF NANOGRAMS INJECTED INTO CHROMATOGRAPH

Detector	Naphthalene	Phenanthrene	Pyrene	Chrysene	Benzo(a)pyrene
Fixed-wavelength at 254 nm	0.16	0.025	0.085	0.046	0.021
Variable-wavelength detectors					
A at 254 nm	4.7	0.33	1.29	0.42	0.28
B at 254 nm	0.8	0.06	0.20	0.10	0.05
C at 254 nm	4.0	0.26	0.9	0.38	0.5
A at optimum	0.14	0.20	0.26	0.19	—
C at optimum	0.21	—	0.3	0.2	—
D at optimum	0.32	—	0.07	0.12	—
Fluorimeters					
E	—	—	—	—	0.02
F	—	—	—	—	0.007
G	—	—	—	—	0.05
Spectrofluorimeter	6.8	0.5	0.11	0.16	0.012

Table 13
DETECTION LIMITS IN TERMS OF CONCENTRATION PRESENT IN THE CELL (ng/mℓ)

Detector	Naphthalene	Phenanthrene	Pyrene	Chrysene	Benzo(a)pyrene
Fixed-wavelength at 254 nm	0.34	0.035	0.14	0.054	0.022
Variable-wavelength detectors					
A at 254 nm	7.8	0.55	2.2	0.47	0.19
B at 254 nm	140.0	10.0	40.0	10.0	4.0
C at 254 nm	6.6	0.43	1.5	0.45	0.35
A at optimum	0.23	0.33	0.43	0.21	—
C at optimum	0.35	—	0.50	0.23	—
D at optimum	0.53	—	0.11	0.13	—
Fluorimeters					
E	—	—	—	—	0.014
F	—	—	—	—	0.005
G	—	—	—	—	0.003
Spectrofluorimeter	0.23	0.8	0.18	0.18	0.008

specific peaks. Since the assumption was made that each peak resulted from a single compound, a fluorescent impurity would cause an error in the quantitative determination.

The detection limit for each PAH was calculated for the solute peaks with a minimum observable peak height defined as equal to a signal to noise ratio of two. These results are presented in Table 16, and it should be noted that subpicogram amounts of acenaphthene and B(k)F were detectable.

Das and Thomas[30] have also reported the determination of picogram and subpicogram amounts of PAHs by the fluorescence detection of HPLC eluates. A Model FS 970 Spectrofluoro Monitor® (Schoeffel Instrument Corp.) was equipped with a low-volume flow cell (5 μℓ), and a continuously variable source of monochromatic excitation energy was provided by a highly stabilized deuterium lamp (190 to 400 nm). Six emission

Table 14
PAH PEAK WAVELENGTHS IN EXCITATION AND EMISSION SPECTRA

Compound	λ_{ex}[a]	Emission peaks	Excitation peaks	λ_{em}[a]
Naphthalene	250	330	280	340
Acenaphthene	275	328	300	340
Fluorene	260	315	265	310
Phenanthrene	295	355, 365	250, 290, 325	360
Anthracene	305	385, 405, 425, 505	250, 350, 370	405
Fluoranthene	305	340, 485	245, 290, 330, 350	480
Pyrene	295	375, 390, 410	245, 290, 325	390
Benz(a)anthracene	295	390, 410	285, 340	410
Chrysene	295	365, 385, 405	270, 315	385
Benzo(e)pyrene	305	395	290, 330	395
Benzo(b)fluoranthene	300	500	280, 330	500
Benzo(k)fluoranthene	295	415, 435	250, 305	410
Benzo(a)pyrene	305	410, 430, 460	270, 295, 370, 390	410
Dibenz(a,h)anthracene	300	395, 430, 440	292, 350	400
Benzo(ghi)perylene	305	340, 420	290, 370, 385	420
Indeno(1,2,3-cd)-pyrene	305	480, 500	250, 300, 360	500

[a] Wavelengths in nm.

Table 15
THREE GROUPS OF PAH AND THE APPROPRIATE COMPROMISE EXCITATION AND EMISSION WAVELENGTHS

λ_{ex} = 280 nm, λ_{em} = 340 nm
- Naphthalene
- Acenaphthene
- Fluorene
- Phenanthrene

λ_{ex} = 305 nm, λ_{em} = 430 nm
- Anthracene
- Fluoranthene
- Pyrene
- Benz(a)anthracene
- Chrysene
- Benzo(e)pyrene
- Benzo(b)fluoranthene
- Benzo(k)fluoranthene
- Benzo(a)pyrene
- Dibenz(a,h)anthracene
- Benzo(ghi)perylene

λ_{ex} = 305 nm, λ_{em} = 500 nm
- Indeno(1,2,3-cd)pyrene

cut-off filters — 370, 389, 418, 470, 550, and 580 nm — were used to select the desired emission wavelengths.

After preliminary experiments to determine the optimum value of the excitation and emission wavelengths for each PAH, detection limits were estimated from a

Table 16
DETECTION LIMIT FOR EACH PAH AS CALCULATED FROM THE SIGNAL-TO-NOISE RATIO OF THE INDIVIDUAL PEAK IN CHROMATOGRAMS

Compound	Detection limit[a] (pg)
Naphthalene	30
Acenaphthene	0.5
Fluorene	12
Phenanthrene	130
Anthracene	120
Fluoranthene	40
Pyrene	75
Benz(a)anthracene	35
Chrysene	70
Benzo(e)pyrene	45
Benzo(b)fluoranthene	3
Benzo(k)fluoranthene	0.3
Benzo(a)pyrene	2.5
Dibenz(a,h)anthracene	4
Benzo(ghi)perylene	9
Indeno(1,2,3-cd)pyrene	8

[a] Compromise wavelengths.

Table 17
DETECTION LIMITS OF STANDARD PAH

Compound	λ_{ex}(nm)	λ_{em}(nm)	Amount (pg)
Fluoranthene	280	>389	0.5
Benz(a)anthracene	280	>389	0.6
Benzo(k)fluoranthene	280	>389	0.4
Benzo(e)pyrene	280	>389	5.1
Benzo(a)pyrene	280	>389	1.1
Dibenz(a,h)anthracene	280	>389	2.3
Benzo(ghi)perylene	280	>389	3.0
Chrysene	250	>370	2.3
Perylene	250	>370	0.6

chromatogram by considering an observable peak to have a minimum signal to noise ratio of two. The limits for nine common PAHs are listed in Table 17. The limits for chrysene and perylene were determined on individual samples at the noted experimental parameters.

Calibration curves were constructed to test the linearity of the detector response. Concentrations from 1 to 100 pg of each PAH in the mixture gave a linear fluorescence response with correlation coefficients between 0.999 and 1.000.

The determination of PAHs using fluorescence detection presents a number of problems since qualitative identification cannot be made without the use of standard samples. The peaks in real samples were identified by the method of standard addition. The identifications were made by use of a differential fluorescence technique in which samples and standards were analyzed under three optimal fluorescence conditions. The

final characterization of peaks was accomplished by determining the peak height ratios at two distinct excitation and emission wavelengths (λ_{ex} 250/$\lambda_{em} > 370$) and comparing with ratios obtained on standards.

Das and Thomas[30] used the principle of selective excitation to determine individual concentrations of chrysene and B(a)A. The use of a column packing capable of resolving the two isomers would be the easiest method for their determination, but a fluorescence method can be attempted when resolution is impossible. Since chrysene exhibits a very weak UV absorption at a wavelength of 280 nm, a very weak fluorescence is emitted with this excitation wavelength. Therefore, B(a)A can be selectively determined in the presence of chrysene up to a B(a)A/chrysene ratio of 0.025 (5 pg B(a)A to 200 pg chrysene) at an excitation wavelength of 280 nm with an emission cut-off wavelength of 389 nm ($\lambda_{em} > 389$ nm). Below this ratio of 0.025, the chrysene contribution becomes significant. This ratio can be reduced to 0.01 if the emission of B(a)A is monitored above 418 nm, but this procedure results in a loss of sensitivity.

It is just as important to be able to measure chrysene alone or in a mixture with B(a)A. Chrysene and B(a)A can both be excited at 250 nm. If the emission is monitored with a cut-off filter so that $\lambda_{em} > 370$ nm, chrysene can be determined in the presence of B(a)A by the use of the following equation:

$$a = b - \frac{c}{d} \qquad (3)$$

where a = peak height due to chrysene at $\lambda_{ex} = 250$ nm and $\lambda_{em} > 370$ nm, b = peak height due to both B(a)A and chrysene at $\lambda_{ex} = 250$ nm and $\lambda_{em} > 370$ nm, c = peak height of B(a)A at $\lambda_{ex} = 280$ nm and $\lambda_{em} > 389$ nm, and d = 3.91, which is the ratio of peak heights of B(a)A at known concentrations at $\lambda_{ex} = 280$ nm $\lambda_{em} > 389$ nm and $\lambda_{ex} = 250$ nm, $\lambda_{em} > 370$ nm. Das and Thomas tested the validity of this equation by a series of experiments in which chrysene/B(a)A ratio varied from 0.36 to 4.36. The constant 3.91 is invariant over changes in HPLC parameters as long as the fluorescence measurements at 250 and 280 nm are made under identical HPLC conditions. Das and Thomas have also applied this mixture analysis to coeluting peaks at mol wt 252; perylene and benzo(*a*)pyrene.

Eisenbeiss et al.[50] have also studied the fluorescence properties of PAHs isolated by HPLC. Optimum excitation and emission wavelengths for the analysis of each component were determined from individual spectra. These data were used to establish limits of detection which were on the nanogram to subnanogram level. For six, WHO PAHs studied, an excitation wavelength of 367 nm and an emission wavelength of 470 nm was considered a good compromise for the entire group.

Fox and Staley[32] have also used fluorescence detection for quantitation of PAHs separated by HPLC. In addition to conventional fluorescence detection of the separated PAHs, Fox and Staley used first and second derivative fluorescence spectroscopic techniques to improve peak resolution. For the case of difficult to resolve chromatographic peaks, such as the chrysene/B(a)A fraction, they employed selective modulation fluorescence techniques. The fluorescence emission spectrum of the HPLC fraction obtained from an airborne particulate sample, which corresponds in terms of retention time to the expected chrysene fraction, is shown in Figure 21. The standard emission spectra of pure chrysene and B(a)A are shown in Figures 22A and B. Modulation of the excitation monochromator at the maximum absorption peak of chrysene will effectively nullify the contribution of chrysene to the peak in Figure 21. The results of such a selective modulation technique applied to the fraction shown in Figure 21 are shown in Figure 23. If the peak in Figure 21 had consisted of only pure chrysene, the result would

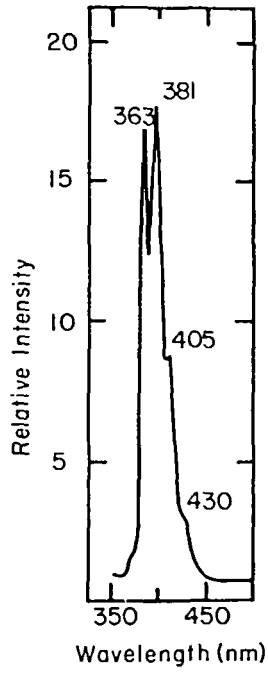
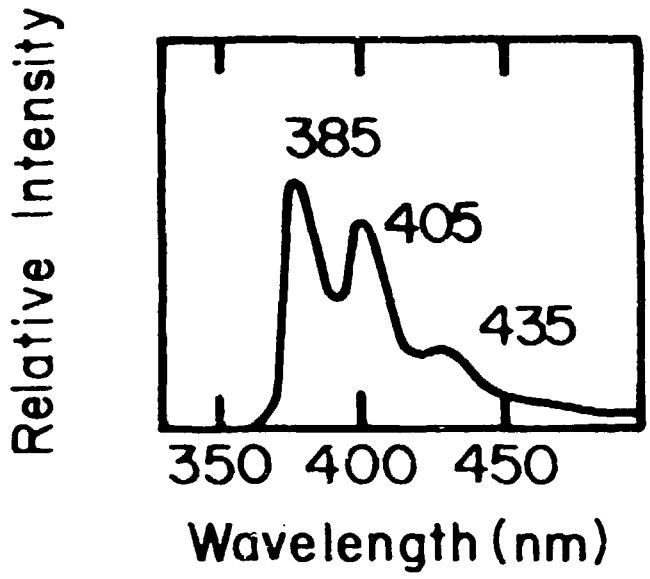


FIGURE 21. Application of selective modulation to enhance the resolution of chrysene and benz(a) anthracene by HPLC. Fluorescence emission spectrum of a fraction from a Baltimore Harbor Tunnel sample consisting of a mixture of chrysene and benz(a)anthracene; λ_{exc}, 298 nm.

be identical to curve 2 of Figure 23 which was obtained a test run on pure chrysene. However, upon application of the technique, curve 1 of Figure 23 resulted. Curve 1 was identified as being identical to the spectrum of pure B(a)A except for an impurity at 434 nm. This impurity was traced to the solvent. In the same fashion, other coeluting PAHs e.g., B(e)P and B(k)F were determined.

D. Future Outlook

As far as detection systems for HPLC are concerned, the reported success in measuring picogram amounts of PAHs is very impressive. The present success in detecting these levels has been accomplished with conventional deuterium, tungsten-halogen excitation, and xenon sources. With the increasing use of lasers as excitation sources in fluorescence studies, it can be expected that HPLC fluorescence detectors will use some form of laser excitation and subpicogram and possibly femtogram (10^{-15}g) levels of PAHs will be measured. There are many reasons to strive for the realization of these lower detection limits. First, evidence from toxicologists and medical researchers is far from conclusive as to the levels of carcinogenic PAHs which are medically significant. Second, many of the more highly active carcinogenic PAHs are of very high molecular weight, e.g., the dibenzanthracenes and dibenzopyrenes. The possible health hazards of even higher molecular weight PAHs, i.e., mol. wt. $>$ 300, are for the most part unknown and unreported in the literature. These PAHs are only very slightly soluble in water and are for the most part undetected at present, even after preconcentration steps. If the concentrations of these PAHs in water systems could be determined, more could be



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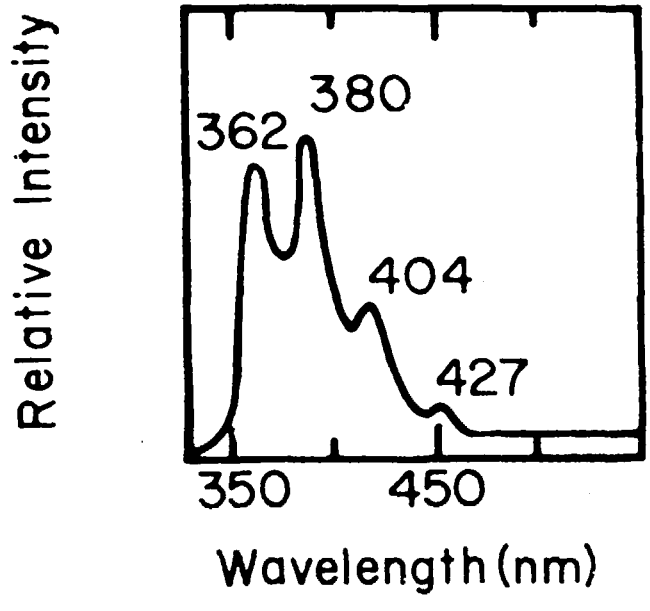


B

FIGURE 22. Fluorescence emission spectra of standard chrysene and benz(a)anthracene samples. (A) Fluorescence emission spectrum of a standard sample of benz(a)anthracene (in methanol); λ_{exc}, 296 nm; (B) fluorescence emission spectrum of a standard sample of chrysene (in methanol); λ_{exc}, 296 nm.

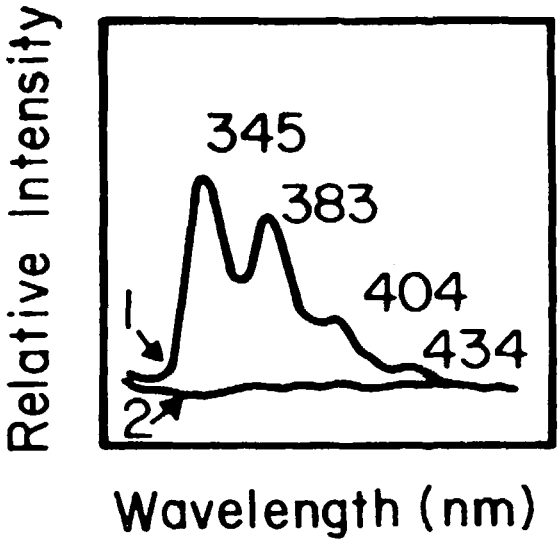


FIGURE 23. Resulting curves from the selective modulation procedure to analyze the mixture of chrysene and benz(a)anthracene. Curve 1: Selectively modulated fluorescence emission spectrum of the same sample whose normal spectrum is shown in Figure 21; excitation modulation at the absorption maxima of chrysene. (w_{ex}, 2.0 mm; w_{em}, 0.5 mm, 1.5 nm/sec, sensitivity, 3 % full scale; J, 1 s). Curve 2: Selectively modulated (i.e., nulled) fluorescence emission spectrum of a standard chrysene sample (whose normal spectrum is shown in Figure 22b) under conditions as in Curve 1.

learned about the possible health effects of these compounds. Finally, most of the error and uncertainty present in the analyses of PAHs in water arises from experimental problems in the extraction and preconcentration steps, and lower limits of detection would make it possible to forego this step. Since HPLC reversed phase analyses are carried out with aqueous mobile phases, the water sample could be directly injected into the liquid chromatograph.

The major drawback of fluorescence detection is the inability of the method to provide qualitative as well as quantitative identification of peaks. Whereas mass spectrometry (MS) and infrared spectroscopy can provide qualitative information to allow identification of individual PAHs, solution fluorescence spectra taken at room temperature are seldom sufficiently detailed to allow unambiguous identifications. Therefore, a standard sample of every component present is necessary in order to confirm the presence of a compound by fluorescence analysis. Matrix isolation techniques can be used to overcome this problem. Fluorescence spectra recorded at 15 K with the PAH dispersed in an inert gas matrix are capable of providing fingerprint-like detail sufficient for identification. However, this technique is at present in the development stage and is not yet capable of online HPLC monitoring. Development work is also in progress on HPLC-MS interfaces[51] which should provide the same degree of information that is presently available from GC/MS systems. Before systems of this type are available to the analyst, the direct introduction of an HPLC eluent into a mass spectrometer must be resolved.

At present, sample cleanup and PAH fractionation are still essential prior to HPLC analysis. Although model solutions can be readily handled, environmental samples are

rarely simple. Since fluorescence detection requires the use of standards, the extent of interpretation of environmental samples is limited by the number of available standards. Coeluting peaks and interfering fluorescence spectra make one-step identifications by fluorescence techniques impossible.

To simplify the interpretation of the results of fluorescence analysis, many of the column and thin layer chromatographic techniques as well as the two-stage HPLC separation of Wise et al.[26] can be employed as a separation step. The degree of cleanup necessary obviously depends on the complexity of the sample. Fresh water lake samples would not be treated in the same way as an oil slick water sample.

HPLC has proven successful in resolving many isomeric PAHs. Further investigations of C_{18} packings should proceed with a view to the development of a packing that will resolve a greater number of isomers. The continuing development of gradient elution techniques in conjunction with reversed phase HPLC applications will enhance the use of HPLC for PAH analyses.

V. GAS CHROMATOGRAPHIC METHODS OF ANALYSIS

A. Introduction

The required properties of a GC stationary phase for separation of PAHs are selectivity for individual compounds and thermal stability over the range of temperatures necessary to elute all components. For packed column GC no material has yet been developed that satisfies both these demands. Despite this drawback, packed column GC has been widely used over the past 20 years for analyses of PAHs. Two major advances in the past several years have now led to a sharp decline in the use of packed column GC. First, the development of HPLC stationary phases selective to PAHs, coupled with the increase in the availability of HPLC gradient elution techniques has led to a shift away from packed column GC to the use of HPLC. Second, a number of difficulties associated with the use of capillary columns in GC have been eliminated. With the increased efficiency of capillary columns, it is now possible to resolve complex mixtures of PAHs with the same stationary phases that have been used in packed GC columns and at lower column temperatures. As an indication of this trend, PAH analyses by GC at Batelle's Columbus Laboratories are being done by capillary column GC. The present status and future outlook of capillary and packed column GC methods for analyses of PAHs are reviewed.

Flame ionization detection is most widely used in PAH analysis. A number of specialized techniques such as gas phase fluorescence and photoionization are in developmental stages and matrix isolation fluorescence and Fourier Transform-IR methods may have future application. The combination of gas chromatography with mass spectrometry is a powerful analytical method. The advantages of such a system, especially when coupled with a computer data system, make this detection system the first choice for PAH analysis.

B. Packed Column Separations

1. Introduction

When environmental or "real" samples are of interest, the laboratory equipped with capillary column GC or fully committed to its introduction should have limited need for packed columns. However, packed columns are being used successfully for analyses of PAHs.

A number of stationary phases have been employed for PAH analyses; among these are SE-30, OV-1, OV-101, OV-7, and Dexsil® 300 and 400. All of these stationary phases have approximately the same selectivity for PAHs. The Dexsil® phases show the best

thermal stability on the basis of low column bleed at temperatures up to 320° C; therefore, packed column applications with non-Dexsil® stationary phases are not covered in this review.

A number of liquid crystal stationary phases exhibit remarkable selectivity towards individual PAHs. However, their applicability is limited to small temperature regions between 200 and 300° C, and this severely limits the application of temperature programming. These stationary phases also exhibit high levels of column bleed, and this results in columns with lifetimes of 1 month or less. Despite these serious disadvantages, these stationary phases can be useful for certain specific applications.

2. Practical Considerations

The selection of a carrier gas, flow rate, solid support, and temperature program are important parameters in packed column separations, but are not the focus of current research. Helium and nitrogen are both equally suitable as carrier gases. For maximum efficiency and the prevention of column deterioration, provisions must be made to reduce the oxygen and water content of the carrier gases. Oxygen scrubbers and molecular sieve traps to remove moisture are commercially available.

The flow rate, u, of the carrier gas can have an affect on column efficiency and this is expressed in the van Deemter equation:[52]

$$H = A + B/_u + Cu \qquad (4)$$

where A, B, and C are constants characteristic of the column, column solutes, stationary phase, and mobile phase, and H is the height equivalent to a theoretical plate. Plots of H vs. u should result in a curve with a minimum H value at the optimum flow rate.

The choice of a solid support is less important than that of a stationary phase, but the support should be chemically inert toward all substances and should have a large surface area that results in uniform liquid phase coverage. The most commonly used support for Dexsil® phases is white diatomite derived from filter-aid and is commercially available as Chromosorb® W-HP, manufactured by Johns-Manville.

Finally, one must incorporate an appropriate temperature program since PAHs include compounds ranging from mol. wt. 128, naphthalene, to mol. wt. 300, coronene. Retention times under isothermal conditions are dependent on the molecular weight, and there is no single temperature at which an isothermal separation of all PAHs is feasible. A temperature range from approximately 80 to 300° C is satisfactory for compounds from naphthalene to coronene. With temperature programming, one obtains a chromatogram with narrow, evenly spaced peaks over the entire molecular weight range, and this is analogous to the result achieved by gradient elution with HPLC. A temperature gradient of 4° C/min is commonly reported in the literature, but little loss in performance results from a gradient of 6 or 8° C/min. An advantage of the latter gradients is a reduced analysis time.

3. Dexsil® 300 Separations

Dexsil® 300, as well as the similar stationary phases Dexsil® 400 and 410, is manufactured by Olin Corporation and is available through Analabs, Inc. (a division of Foxboro Analytical). The structure of the phases, shown in Figure 24, consists of metacarborane units (carborane icosahedral cage structures) connected by siloxane units.[53] The physical properties of the Dexsil® series are listed in Table 18. The most important feature is the high temperature stability which is unique among stationary phases used in GC determination of PAHs. As a consequence of this thermal stability, the Dexsil®-packed columns exhibit a very low bleed rate. After 72 hr of conditioning at 380° C, a mass spectrum of the background and column bleed indicated only a small number of fragments.[54]

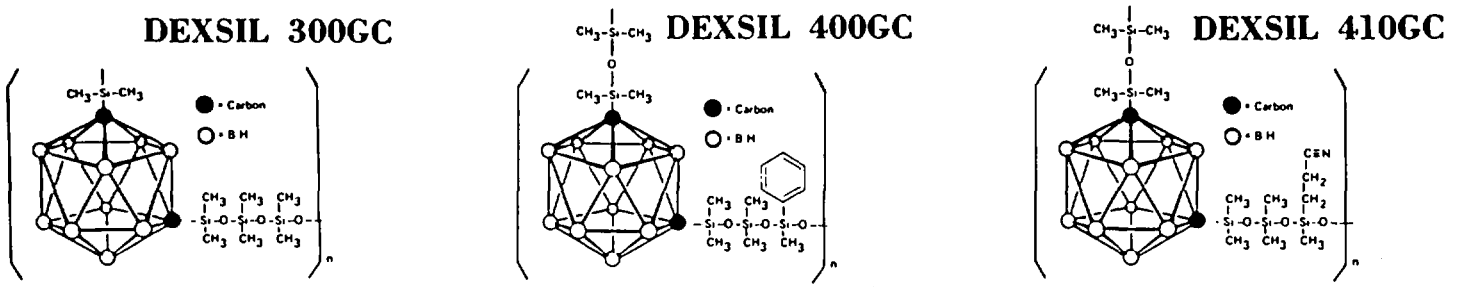


FIGURE 24. Structures of the Dexsil® series of GC stationary phases.

Table 18
CHARACTERISTICS OF DEXSIL® STATIONARY PHASES

Dexsil® 300 GC (carborane-methyl silicone) (50—400+° C)

Dexsil® 400 GC (carborane-methylphenyl silicone) (20—375° C)

Dexsil® 410 GC (carborane-cyanoethyl methyl silicone) (20—360° C)

Dexsil® 300, with proper conditioning and on a carefully prepared support (no trace base, or Fe), can be used to 400+° C and is the most thermally stable stationary phase

Dexsil® 400 is only slightly more polar than Dexsil® 300, but is less viscous and more efficient

Dexsil® 410 is the most polar of the carborane silicones

Properties

	Dexsil® 300 GC	Dexsil® 400 GC	Dexsil® 410 GC
Solubility	Soluble in ether, dichloromethane, chloroform, aromatic solvents; insoluble in water, alcohols		
Physical appearance	Waxy, white solid melting point 30—38° C	Opaque, viscous fluid	Opaque, viscous fluid
Siloxane-carborane ratio	4 to 1	5 to 1	5 to 1
Stability	Stable to most chemicals except strong bases		
Average molecular weight	16,000—20,000	12,000—16,000	9000
Temperature: min/max	50—450° C (500° C, limited time)	20—375° C	20—360° C

Thomas et al.[55] and Lao et al.[56] have studied the PAH fraction from a wide variety of environmental samples such as airborne particulate matter, coal tar, and wood preservative sludge using packed column GC with Dexsil® 300 and 400. With a packing of 6% Dexsil® 300 on 800/100 mesh Chromosorb® W-HP, 124 PAHs present in air samples were identified with more than 100 compounds sufficiently resolved for quantitative measurement.[56] However, with this column isomeric PAHs, e.g., B(*a*)P and B(e)P, anthracene and phenanthrene, and chrysene and triphenylene were not resolved. It was found, however, that 6% Dexsil® 400 packings provided a modest resolution of B(*a*)P from B(e)P, and chrysene from B(a)A.[56] In further studies,[58] Lao's group studied various other environmental samples using 3% Dexsil® 300 packings and obtained similar results.

Strosher and Hodgson[59] determined PAHs in waters and sediments from the Great Lakes after separation on a GC column packed with 3% Dexsil® 300 on 80/100 mesh Chromosorb® W/AW. The usual difficulties were encountered in the separation of isomeric PAHs.

Strand and Andren[60] compared Dexsil® 300 to liquid crystal stationary phases for separations of PAHs. Their separations on a 3% Dexsil® 300 column are shown in Figure 25. They point out that although the Dexsil® 300 packed column has the necessary thermal stability required to elute six- and seven-membered rings, it is incapable of resolving isomeric PAHs.

In our studies of separations of PAHs with a 3% Dexsil® 300 packed column, we have noted the excellent thermal stability necessary to elute higher molecular weight compounds such as coronene. A temperature gradient of 8°/min from 30° to 330° C was employed. The level of column bleed at 330° C was not serious. The complete resolution of an 18-component mixture of PAHs is illustrated in Figure 26. The mixture does not include all of the isomers at MW:228 and 252 which, if present, would coelute with B(a)A and B(*a*)P. However, a number of separations should be noted, e.g., acenaphthylene and acenaphthene, phenanthrene and carbazole, 2- and 9-methylanthracene, pyrene and fluoranthene, B(a)A and naphthacene, and D(a,h)A and B(ghi)P. In addition, PAHs of molecular weight greater than 252, e.g., dibenz(a,b)anthracene and coronene, are eluted

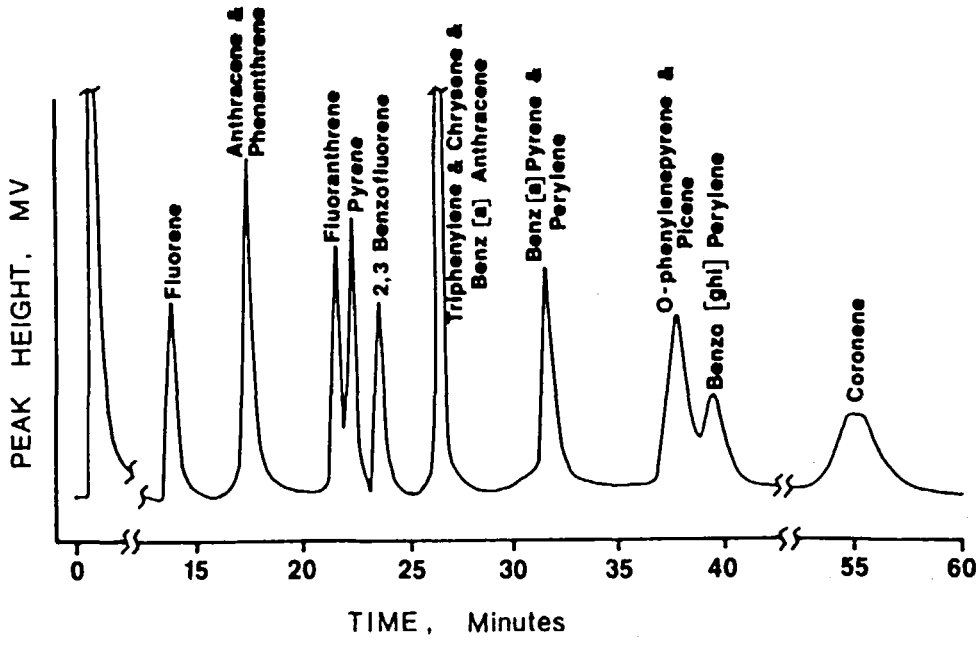


FIGURE 25. Chromatogram of 16 PAH mixture on Dexsil® 300. Column: 15 ft × 0.125 in. O.D. silanized glass. Packing: 3% (w/w) Dexsil 300®. Conditions: oven 110 to 320°C, 4°C/min; on-column injection; detector, 300°C; flow rate, 51 mℓ/min. Concentrations: 100 ppm.

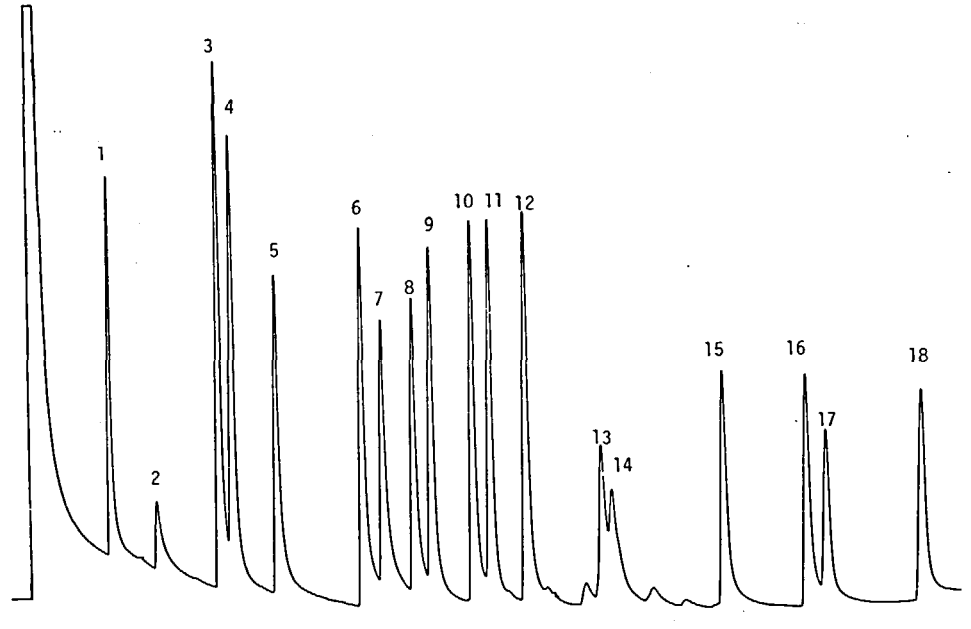
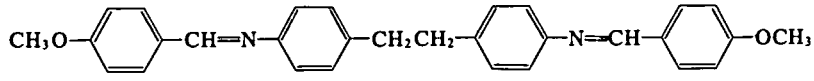


FIGURE 26. Gas chromatogram of a synthetic mixture of 18 PAHs on a Dexsil 300® packed column. Column: 6 ft × 1 ft 8 in. O.D. stainless steel. Packing: 3% Dexsil 300® gc on Chromosorb® W-HP (100/120 mesh). Conditions: initial oven temperature, 80°C; program rate, 8°C/min.; final temperature, 330°C; injector, 275°C; detector, 330°C; flow rate, 35 mℓ/min. He. Concentration approximately 100 ppm. Peak legend: (1) naphthalene, (2) 2-methylnaphthalene, (3) acenaphthylene, (4) acenaphthene, (5) fluorene, (6) phenanthrene, (7) carbazole, (8) 2-methylanthracene, (9) 9-methylanthracene, (10) fluoranthrene, (11) pyrene, (12) benzo(b)fluorene, (13) benz(a)anthracene, (14) naphthacene, (15) benzo(a) pyrene, (16) dibenz(a,h)anthracene, (17) benzo(ghi)perylene, (18) coronene.

as sharp peaks, and coronene is eluted in *less* than 30 min. Retention times for the PAHs studied are listed in Table 19. These values were reproducible to less than 1% within a single day and less than 2% over a period of months with the same column and operating parameters. From these results it can be seen that Dexsil® 300 is unsuitable as a GC column packing for the resolution of isomeric PAHs. However, when the separation of the isomers at MW:228 and 252 is not important, Dexsil® 300 is a good packing since it has the necessary thermal stability to elute coronene in less than 30 min and will provide reproducible retention times for PAHs ranging from naphthalene to coronene.

4. Liquid Crystal Stationary Phases

The liquid phases developed by Janini et al.[61-64] at the National Cancer Institute in Maryland exhibit remarkable selectivity towards PAHs, including isomeric species. *N,N'*-bis(*p*-methoxybenzylidene)-α,α'-bi-*p*-toluidine, as well as the *p*-butoxy, *p*-hexyloxy, and *p*-phenyl analogs of the above have all been investigated as stationary phases for PAH separations. The structure of the methoxy liquid crystal is



and the others are similar, with the replacement of the methoxy groups by another group. The shorthand symbol, BMBT, is given to the methoxy liquid crystal with BBBT, BHxBT, and BPhBT used to denote the analogous versions.

Separation on the liquid crystal phase is based on one essential characteristic of the PAH. The various PAHs, including the structural isomers, differ in their length to width ratio, or rod-like character. The more rod-like the PAH, the greater is the interaction between the PAH and the nematic solvent. As an example, anthracene is retained more strongly than phenanthrene, thereby providing a separation of the two isomers.

These stationary phases have a number of practical limitations. First, separations occur only when the liquid crystal is in the nematic phase. In many cases, this phase exists in a narrow temperature region, and this limits the effective application of temperature programming. In addition, the lower temperature limit of the nematic region is often too high (180 to 200° C) to permit determination of lower molecular weight PAHs since these co-elute with the solvent at these temperatures. Second, the entire nematic region may not be practically accessible because of the poor thermal stability of the liquid crystal phases. Column bleed is often high throughout the entire workable range and tends to become excessive at the higher temperatures. As a result of this poor thermal stability, retention times often change drastically over a period of weeks. Third, the efficiency of columns packed with liquid crystal phases is low (i.e., peak shapes are generally broad) and this limits the total number of compounds which can be resolved. However, despite these serious disadvantages, the liquid crystal phases are the only packings truly able to differentiate between isomeric PAHs at this time.

BMBT was the first liquid crystal to be studied by Janini et al.[61] The nematic region is from 181 to 320° C. However, after 72 hr of operation of the column at 300° C, a loss of 10% of the stationary phase occurred; an upper temperature limit of 265° was considered necessary to reduce column bleed. The separation of a 16-component PAH mixture on a 2.5% (w/w) BMBT-Chromosorb® W-HP column packing is illustrated in Figure 27. The separation of isomers is remarkable. The following isomeric PAHs were baseline resolved: anthracene/phenanthrene, fluoranthene/pyrene, triphenylene/B(a)A/chrysene/naphthacene, and B(k)F/B(*a*)P/B(e)P/perylene. Since B(k)F has the largest length to width ratio of all the benzofluoranthene isomers, others which may be present in environmental samples would be expected to elute earlier and not interfere with the benzopyrenes and perylene.

Table 19
RETENTION TIMES FOR 18 PAH STANDARD ON A 3% DEXSIL® 300 GC COLUMN—CHROMATOGRAPHIC CONDITIONS IDENTICAL TO FIGURE 26

Compound	Average retention time (sec)[a]	Standard deviation (sec)[b]
Naphthalene	151	3.7
2-Methylnaphthalene	236	4.1
Acenaphthylene	355	3.5
Acenaphthene	383	3.3
Fluorene	463	4.1
Phenanthrene	626	5.8
Carbazole	665	5.8
2-Methylanthracene	725	6.1
9-Methylanthracene	762	4.2
Fluoranthene	842	5.3
Pyrene	878	5.4
Benzo(b)fluorene	944	5.2
Benz(a)anthracene	1091	7.3
Naphthacene	1107	7.0
Benzo(a)pyrene	1324	6.4
Dibenz(a,h)anthracene	1480	5.8
Benzo(ghi)perylene	1516	6.0
Coronene	1700	7.8

[a] Average of five replicate runs.
[b] Standard deviation based on five runs.

In an effort to increase column lifetimes by a reduction in column bleed, Janini et al. developed the *p*-butoxy analog BBBT.[62] Whereas the BMBT column lost about 16% of its liquid phase content when operated at 260° C for 170 continuous hours, the losses for the BBBT column were sufficiently low that the HETP was reproducible to 3% as measured on the B(a)A peak. The nematic range of BBBT is 188 to 303° C but the effective upper temperature is 265° C. A typical separation of 13 PAHs with BBBT is shown in Figure 28; these separations are as good as those achieved with BMBT shown in Figure 27.

For PAHs with mol. wt. >252, Janini et al.[63] prepared two new liquid crystals, BHxBT and BPhBT, the *p*-hexyloxy and *p*-phenyl analogs of BMBT. The theoretical plate count for chrysene was reproducible to within 5% after 100 hr of continuous operation at 275° C for the BPhBT and BH × BT phases. It was found that 1-in. long plugs of a preconditioned (5 to 6%) SE-30 packing at both ends of the column increased column lifetimes with no loss in performance. The separation of five 22-carbon PAHs at 280° C under isothermal conditions are illustrated in Figure 29. The dibenzanthracene isomers were resolved from each other as well as from B(ghi)P and picene. Five 24-carbon PAHs, four dibenzopyrene isomers, and coronene were baseline resolved (Figure 30), demonstrating that PAHs of molecular weight over 300 can be separated on liquid crystal phases.

Strand and Andren[60] have also examined separations of PAHs on BBBT and BPhBT stationary phases. Under operating conditions essentially identical to those used by Janini's group, the chromatograms of a 15 PAH mixture shown in Figure 31 and 32 were obtained. Separations were excellent, but the efficiencies of the columns were poor compared to Janini's columns. It was noted that retention times increased by more than

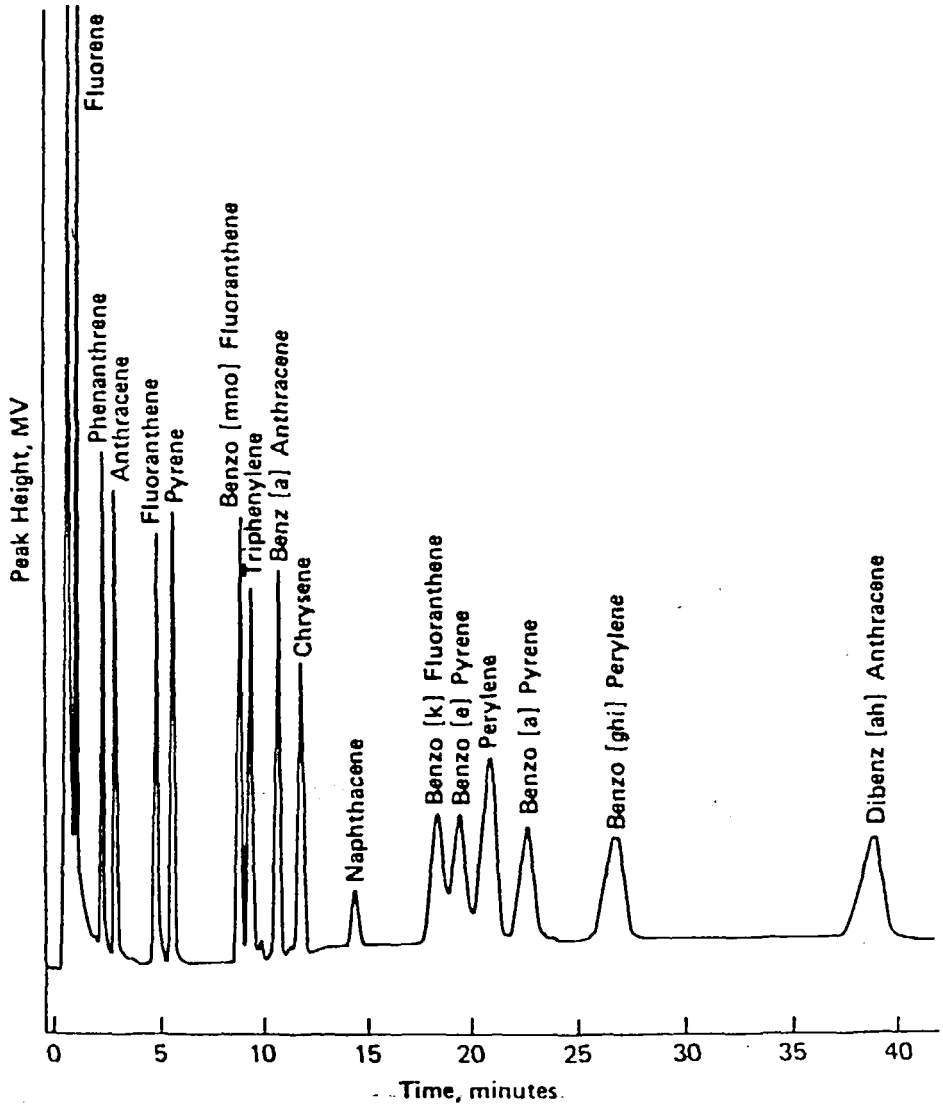


FIGURE 27. Chromatogram of a synthetic mixture of 16 polycyclic aromatic hydrocarbons of wide molecular weight range. Column: 4 ft × 0.125 in. O.D. stainless steel. Packing: 2.5% (w/w) BMBT. Conditions: initial oven temperature, 185°C; initial hold, 2 min; program rate, 4°C/min; final temperature, 265°C; injector, 265°C; detector, 265°C; flow rate, 40 mℓ/min. Concentration ranges from about 0.1 μg of phenanthrene to about 0.2 μg for benzo(ghi)perylene, except for naphthacene, which was less, but undetermined.

10% when the BBBT column was operated continuously for 1 week at 190° C. The BPhBT column maintained reproducible retention times for 1 month while in continuous operation at 270° C.

When HPLC or capillary column GC methods are unavailable, packed column GC with liquid crystal stationary phases are a useful alternative. Unfortunately, no liquid crystal column is able to handle the entire molecular weight range of compounds present in an environmental sample. Therefore, two columns in series can be used — e.g., a Dexsil® 300 column followed by a BPhBT column. It is unfortunate that a class of stationary phases that is so highly selective to PAHs should have serious limitations in

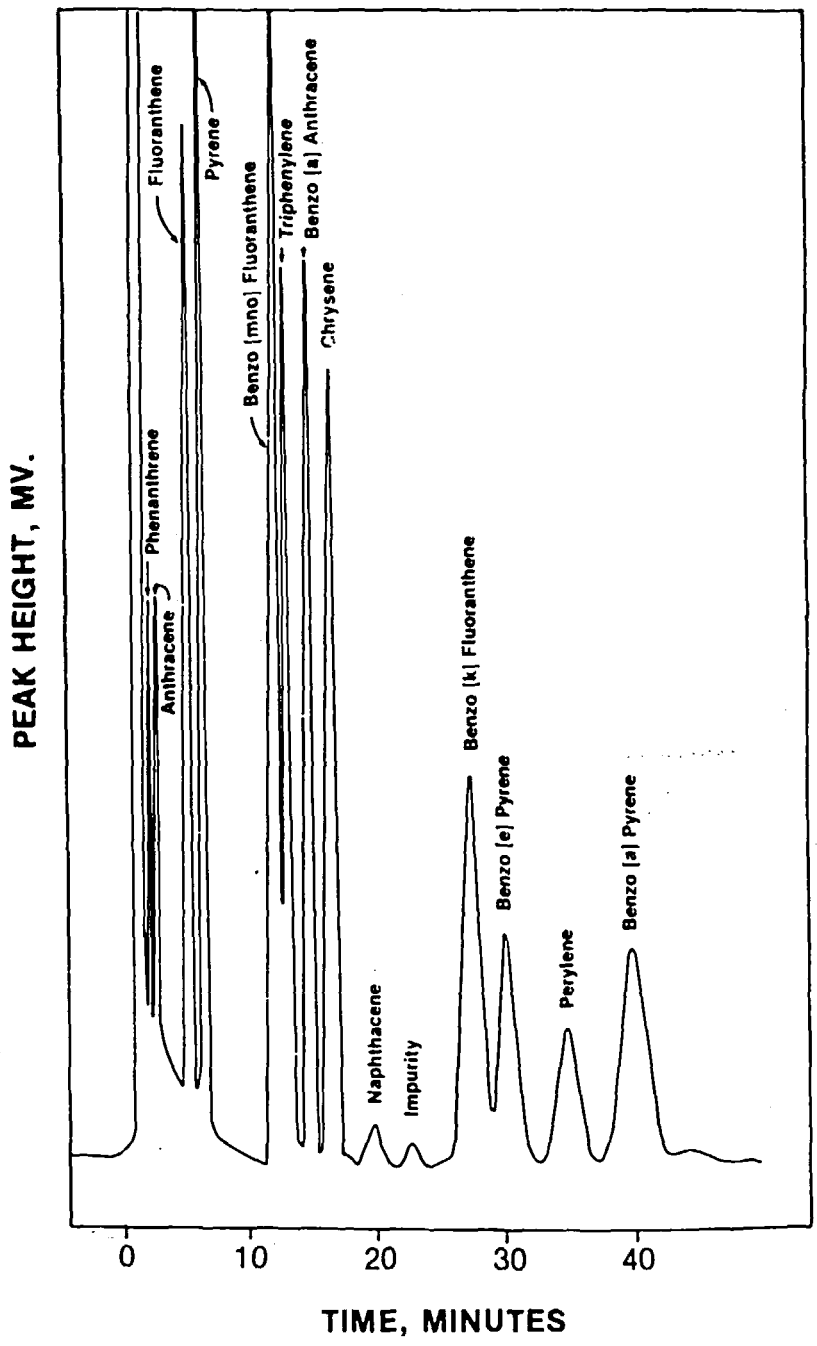


FIGURE 28. Chromatogram of a synthetic mixture of 13 polycyclic aromatic hydrocarbons of wide molecular weight range. Column: 6 ft × 0.125 in. I.D. Supelco® glass. Packing: 2.5% (w/w) BBBT. Conditions: initial oven temperature, 210°C; initial hold, 3 min; program rate, 4°C/min; final temperature, 255°C; flow rate, 24 mℓ/min; electrometer setting, 16×10^{-11}.

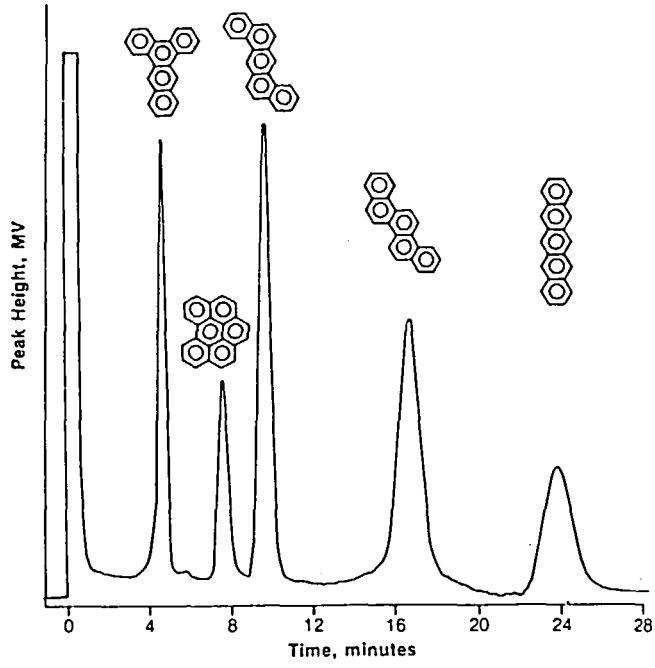


FIGURE 29. Chromatogram of five 22-carbon PAHs on BPhBT at 270°C. Column: 2 ft × 2 mm I.D. glass. Packing: 2.5% (w/w). Conditions: injector and detector, 280°C; flow rate, 30 mℓ/min. Compounds in order of elution are: 1,2,3,4-dibenzanthracene (dibenz-(a,c)anthracene), 1,12-benzperylene (benzo(ghi)perylene), 1,2,5,6-dibenzanthracene (dibenz(a,h)anthracene), 1,2,7,8-dibenzphenanthrene (picene), and 2,3,6,7-dibenzanthracene (pentacene).

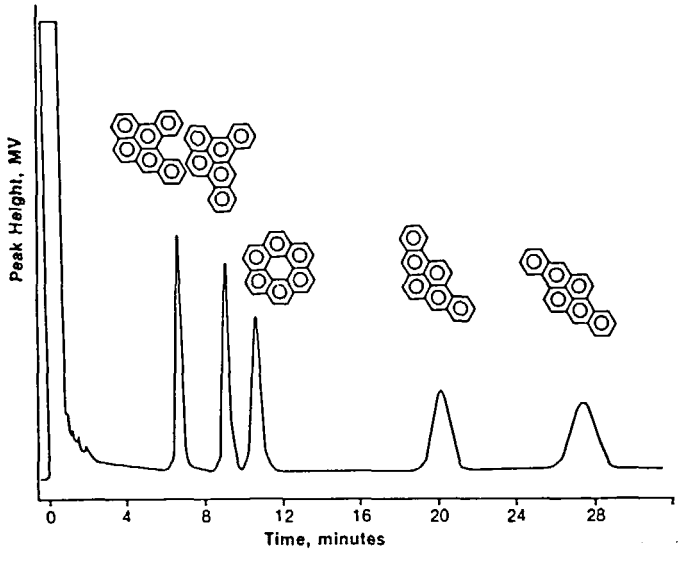


FIGURE 30. Chromatogram of five 24-carbon PAHs on BPhBT at 290°C. Column: 2 ft × 2 mm I.D. glass. Packing: 2.5% (w/w) BPhBT. Conditions: injector and detector, 290°C; flow rate, 50 mℓ/min. Compounds in order of elution are 4,5,6,7-dibenzpyrene (dibenzo(def,p)chrysene, 4,5,7,8-dibenzpyrene, coronene, 2,3,6,7-dibenzpyrene (benzo(rst-)pentaphene), and 1,2,6,7-dibenzpyrene.

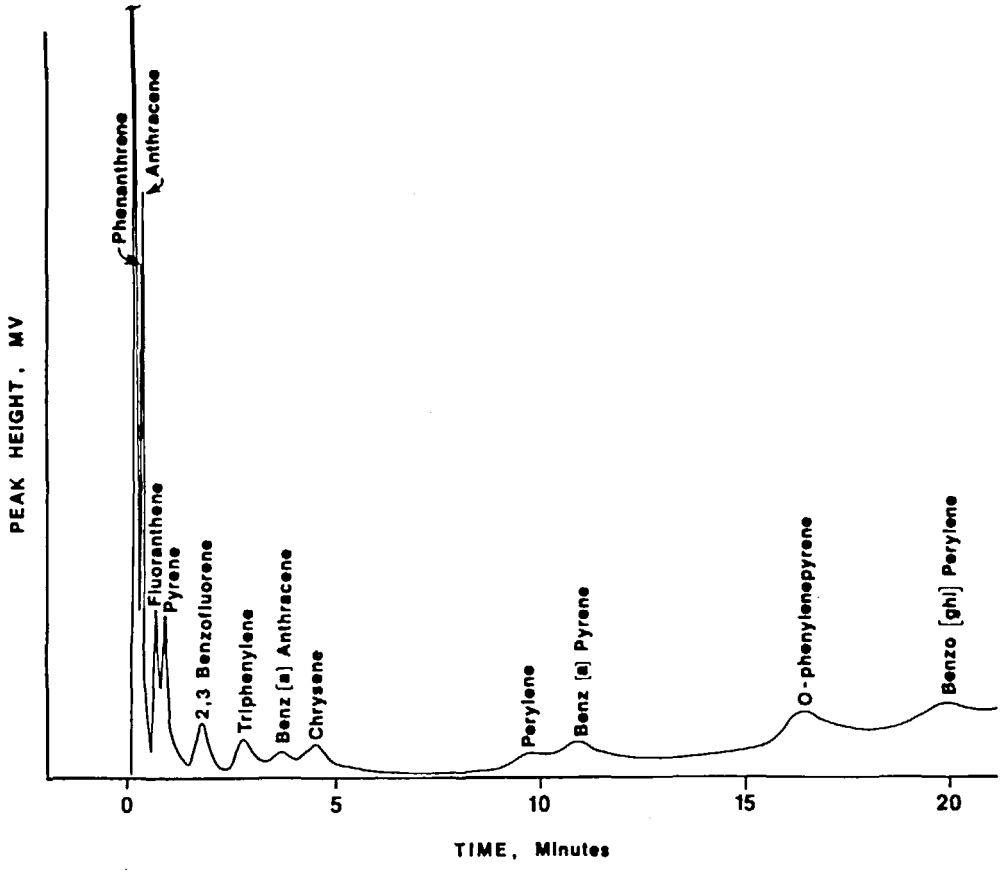


FIGURE 31. Chromatogram of 15 PAH mixture on BBBT. Column: 4 ft × 0.125 in. O.D. silanized glass. Packing: 2.5% (w/w) BBBT. Conditions: oven 190 to 270°C, 2-min hold, 4°C/min; on-column injection; detector, 250°C; flow rate, 40 mℓ/min. Concentrations; 100 ppm.

terms of thermal stability, column efficiency, and wide molecular weight range applicability. Unless further research in this area overcomes these problems, it is highly unlikely that liquid crystal phases will be widely used.

C. Capillary Column Gas Chromatography

1. Introduction

The development of capillary column GC has had a significant impact on methods of analysis of environmental samples. Although introduced more than two decades ago, recent instrumental advances have made it possible to take greater advantage of this technique. Novotny[65] has reviewed the history, analytical methodology, and applications of the entire field of capillary GC. Grob and Grob[66] have thoroughly discussed the technical difficulties of introducing glass capillary GC into the laboratory. The advantages and disadvantages of glass capillary GC and packed column GC as discussed by Grob and Grob[66] and Cram and Yang[67] are summarized here.

Glass capillary and packed column GC were compared by analyzing the same lake water extract by each method.[66] OV-1 was used as the stationary phase in both columns. Because Grob and Grob have been using glass capillary columns in their laboratory since the mid-60s and are expert in their preparation, the capillary columns used in this comparative study were prepared in their own laboratory. To avoid bias, the packed

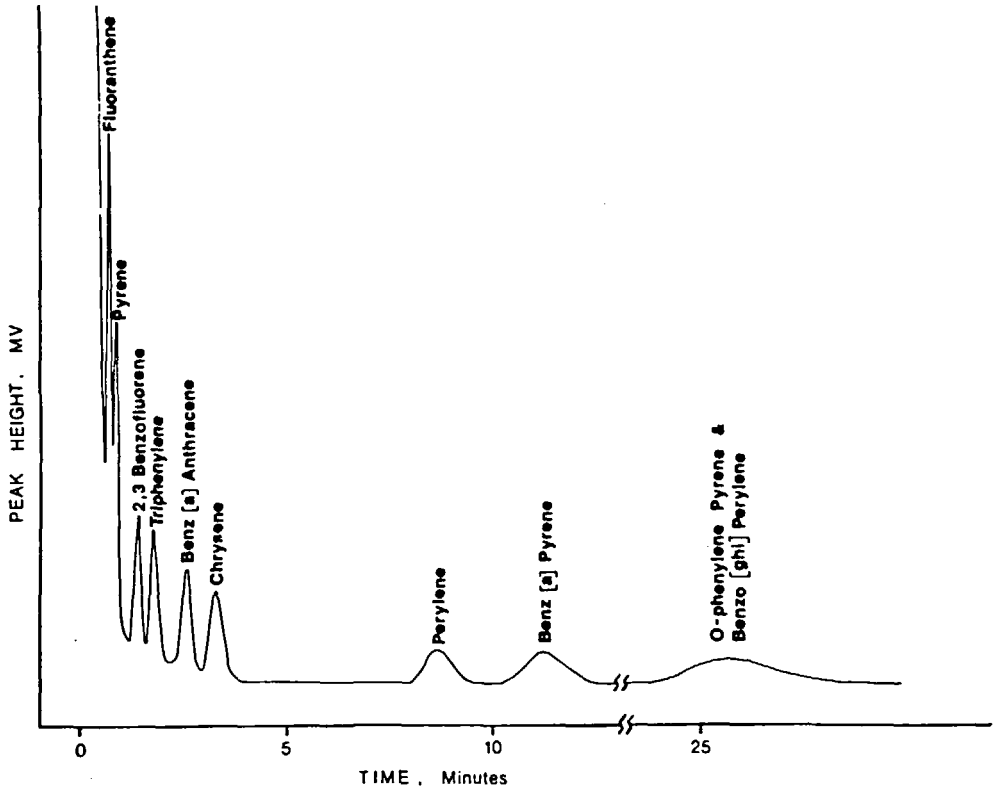


FIGURE 32. Chromatogram of 15 PAH mixture on BPhBT. Column: 6 ft × 0.125 in. O.D. silanized glass. Packing: 2.5% (w/w) BPhBT. Conditions: oven 270°C; on-column injection; detector, 250°C; flow rate, 20 mℓ/min. Concentrations: 100 ppm.

columns were supplied by an expert not associated with their laboratory. The analytical conditions for the packed and capillary columns are listed in Table 20 and the chromatograms obtained from the two columns are shown in Figure 33.

The capillary column was 12 times longer than the packed column and contained less than 2% of the stationary phase of the packed column. The separation efficiency was higher for the capillary; 490 peaks were observed as compared to 118 for the packed columns. It should be noted that it required many trials to achieve this degree of separation with the packed column, whereas the capillary column separation was "routine".[66]

The advantages of glass capillary GC have been listed by Cram and Yang.[67] The glass capillary columns perform more reproducibly than packed columns and baseline stability and resolution are excellent. The peaks obtained with capillary columns are narrower, with essentially triangular symmetry which facilitates quantitation. Since the stationary phase is not coated on a support, the permeability of the capillary column is very high so that longer columns can be used. This added length increases the efficiency of the capillary column and even with this added length, the analysis time is reduced. Because of the increased efficiency, the choice of stationary phase is, in general, not as critical as for packed columns but in spite of this, several isomeric PAHs have yet to be resolved.

An important problem of current interest is the activity of the glass capillary surface. As a result of the composition of glass and its metallic oxide content, many polar organic

Table 20
COMPARISON OF COLUMN CONDITIONS WITH OV-1 IN PACKED AND CAPILLARY COLUMNS

		Capillary	
	Packed	Narrow bore	Wide bore
Length	3 m	35 m	60 m
Inner diameter	2 mm	0.28 mm	0.60 mm
Coating	3% OV-1 on Gaschrom® Q, 80—100 mesh	OV-1, film 1.0×10^{-5} cm	OV-1, film 1.0×10^{-5} cm
Liquid-phase load	120 mg	1.5 mg	14 mg
Carrier gas flow	He, 10-22 mℓ/min (programmed)	H_2, 2.2 mℓ/min	H_2, 14 mℓ/min
Temperature range	50—200° C	25—170° C	25—200° C
Temperature program rate	2° C/min	3.5° C/min	2.5° C/min
Analysis time	90 min	45 min	65 min
Sample size	0.15 μℓ direct	0.6 μℓ, splitting 1:25	0.6 μℓ, splitting 1:8
Attenuation	× 32	× 4	× 16
Number of peaks	118	490	320

solutes will adsorb onto the capillary walls and this results in peak tailing with oxygen and nitrogen functional groups. Although PAHs in general do not suffer from this problem, environmental samples contain numerous oxygen- and nitrogen-substituted PAHs which elute in the same region as PAHs. A number of procedures have been developed for the reproducible deactivation of glass capillaries. The recent introduction of fused silica (about 100% SiO_2) capillaries may eliminate the need for exhaustive deactivation pretreatments.

2. Deactivation of Glass Capillary Columns

Glass is generally chosen as the material for the construction of capillary columns because it is inert compared to other common column materials such as copper, stainless steel, and nickel. However, glass directly from the manufacturer is not suitable without some form of pretreatment. The surface of the glass can contain an appreciable percentage of metallic and boron oxides in addition to acidic silanol sites, depending upon whether soda-lime or Pyrex® glass is involved. These surfaces can be quite "active" leading to problems such as: (1) peak tailing, (2) solute losses, and (3) stationary phase decomposition. These problems are particularly severe when polar solutes are analyzed on nonpolar stationary phases and when higher column temperatures necessary for high molecular weight compounds are used.

Some of the methods which have been developed to deactivate glass surfaces include silanization, pretreatment with thin-layers of nonextractable polymers, and acid leaching. The degree of column deactivation is usually judged by the chromatographic performance on test mixtures of solutes of widely ranging polarity. Recently,[68] Auger Electron Spectroscopy® (AES) was employed in an effort to relate elemental surface concentrations on the glass capillary to the degree of the column activity as judged by chromatographic performance.

Lee et al.[68] have studied the effects of various acid leaching procedures for deactivating the surfaces of glass capillaries. Two basic types of glass were examined, a Kimble® R6 Flint glass (soda lime) and a Corning® Pyrex® (7740) borosilicate glass. The bulk

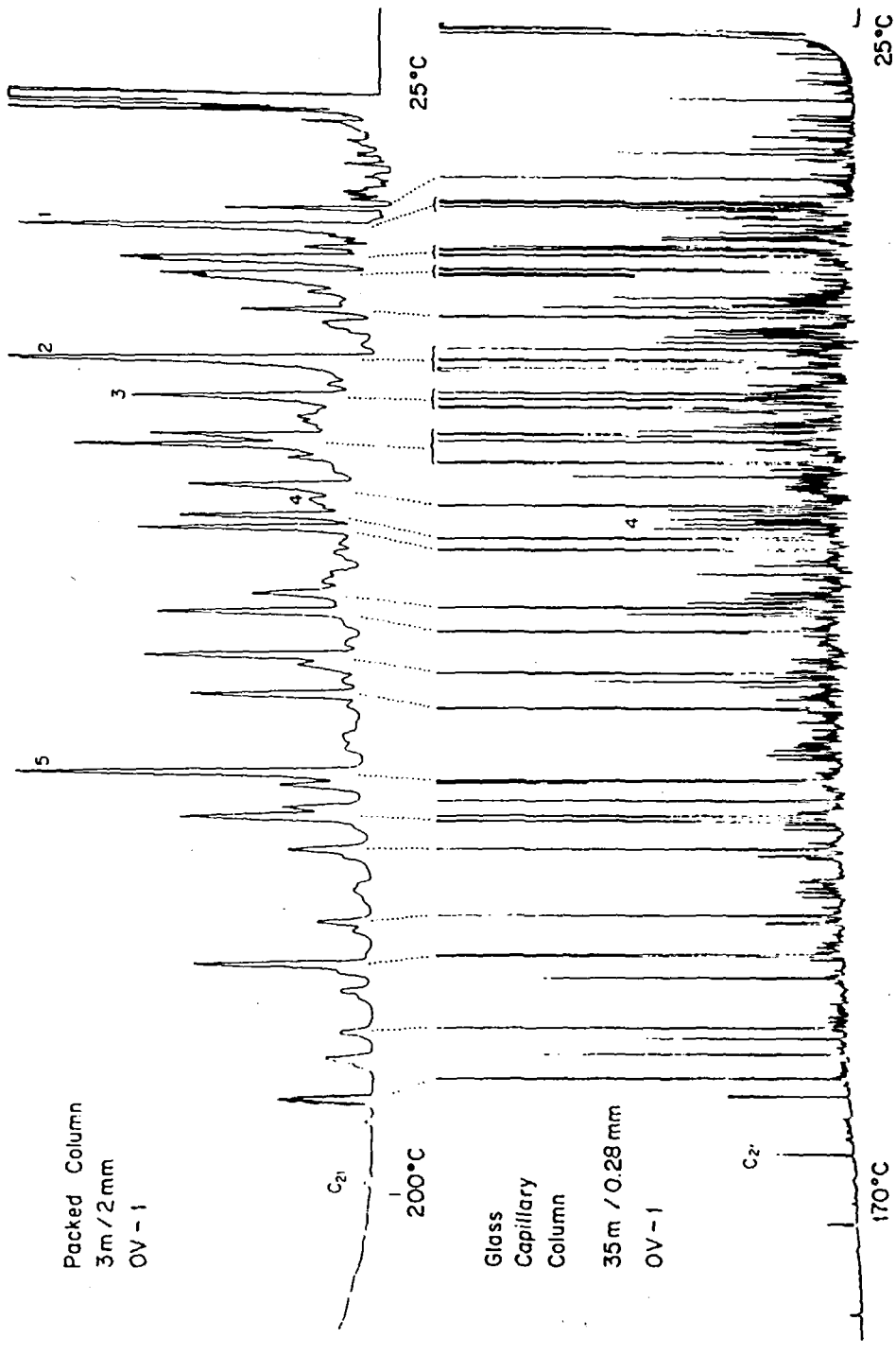


FIGURE 33. Comparison of typical separation from packed and capillary columns. Sample (extract from river water) and liquid phase identical for both columns. For column characteristics and operating chromatographic parameters, see Table 20. Dotted lines indicated corresponding sample components.

Table 21
BULK GLASS COMPOSITIONS

Elements	Composition as Oxide (%)	
	Kimble R6 Flint®	Corning Pyrex 7740®
Si	67.7	81
Na	15.6	4
Ca	5.7	0.5
Mg	3.9	—
Al	2.8	2
Ba	2.0	—
B	1.5	13
K	0.6	—

Table 22
EXPERIMENTAL TREATMENT CONDITIONS

Column number	Glass type	Pretreatment	Leach solution[a,b]
1	Kimble R6®	—	88% Formic acid
2	Kimble R6®	—	19% Hydrochloric acid
3	Kimble R6®	—	Distilled water
4	Kimble R6®	—	19% Hydrochloric acid (100° C)
5	Kimble R6®	Helium purge at 300° C for 24 hr Gaseous hydrochloric acid for 1 hr at 300° C Oven heat at 350° C for 2 hr	88% Formic acid
6	Kimble R6®	Identical to Column 5	19% Hydrochloric acid
7	Kimble R6®	Identical to Column 5	Distilled water
8	Corning Pyrex®	—	19% Hydrochloric acid
9	Corning Pyrex®	—	19% Hydrochloric acid (100° C)

[a] 8mℓ of leach solution was used under nitrogen pressure, for a period of 27 hr. This was followed by a 2mℓ distilled water rinse and subsequent drying with helium.

[b] Unless indicated in parenthesis, the wash was done at room temperature (25° C).

compositions of the glasses are listed in Table 21. The capillary columns were drawn by a Shimadzu Model® GDM-1 glass drawing machine to an internal diameter of 0.28 to 0.30 mm, then broken into 4-in. lengths prior to treatment. The various deactivation procedures are outlined in Table 22. The elemental surface concentrations of the deactivated capillaries as determined by AES spectroscopy are listed in Table 23.

Lee et al.[68] have observed over a period of years that the removal of metallic and boron oxides from the glass surface improved the chromatographic performance of capillary columns and gauged the effectiveness of various deactivation procedures by the degree to

Table 23
CAPILLARY ELEMENTAL SURFACE CONCENTRATION AFTER TREATMENT

Column number	Surface concentration (%)						
	Si	C	O	Na	Ca	Cl	B
1	7	27	52	11	3	—	—
2	9	23	54	11	2	0.5	—
3	8	26	51	11	3	—	—
4	14	29	58	—	—	—	—
4[a]	18	18	64	—	—	—	—
5	12	27	55	4	1	1	—
6	9	35	44	9	1	2	—
7	9	33	45	10	1	2	—
8	11	26	62	—	—	1	2
9	15	23	63	—	—	—	—
9[a]	14	28	58	—	—	—	—

[a] Indicated columns are heat-treated at 250° C for 12 hr under helium carrier gas flow and analyzed again by AES.

which a "pure silica, SiO_2" surface was obtained. The AES results indicated that the most effective treatments for the Kimble® glass were hydrochloric acid leaching at 100° C and gaseous hydrochloric acid/formic acid leaching. In the first procedure, an essentially pure silica surface resulted, while in the second, the sodium and calcium oxide contents were reduced to near zero. The boron content of Corning® Pyrex® was also reduced to "zero" by leaching with hydrochloric acid at 100° C. Chromatograms of C_5 through C_{12} *n*-alcohols obtained on a nonpolar OV-101 Pyrex® capillary column deactivated in this manner displayed a low degree of peak tailing.[68] Samples containing ketones, amines, and free sterols were also successfully analyzed. In addition, this column exhibited excellent thermal stability, i.e., low bleed up to a temperature of 300° C.

Lee et al.[68] noted that difficulties exist in the deactivation process. First, the reproducibility of the various procedures were found to vary in an unpredictable manner. They suggest that investigation of the deactivation of surface silanol groups is necessary and that surface coating procedures might be useful in this respect. Second, it was found that a high percentage (about 20 to 30%) of the elemental surface content was adsorbed carbon which interferes with the deactivation process. They pointed out the need to prevent this carbon contamination, but noted the difficulty of accomplishing this in a laboratory environment. They did not discuss the important question of stationary phase stability before and after deactivation.

Meuser et al.[69] at Battelle's Columbus Laboratories have also developed an acid leaching deactivation procedure for the preparation of thermally stable high-temperature glass capillaries. The leaching was accomplished with 20% hydrochloric acid at 180° C for 16 hr under reduced pressure. Following this step, the acid was washed out and the column heated to 280° C at reduced pressure for 24 hr. Silylation with a 20% solution of diphenyltetramethylsilazone at 400° C for 8 hr completed the treatment. Glass capillary columns treated in this manner and coated with SE-52 were used at temperatures up to 320° C for PAH separations. No results on column bleed levels were given.

Active glass capillary surfaces can catalyze the decomposition of the stationary phase at the high column temperatures necessary to elute the high molecular weight PAHs. Schomburg et al.[70-72] have investigated this problem by focusing on three parameters

which affect the level of column bleed; (1) type of glass (alkali or borosilicate), (2) surface properties after deactivation and roughening treatments, and (3) type and origin of the methylsilicone stationary phases.

The thermal stability of the capillary columns over extended time periods at high temperatures must be assessed on the basis of several factors: (1) capacity ratios of selected standard compounds, (2) separation efficiency in terms of theoretical plates per meter, (3) extent of surface deactivation as gauged by tailing measurements, and (4) polarity in terms of retention indices of standard compounds.[70] For their studies, borosilicate and alkali glass capillary columns of similar length and internal diameter coated with OV-101 and SE-30 were used. These columns were preconditioned by operating at 280° C for about 12 hr at carrier gas flow rates of 1 mℓ / min. The bleed levels were measured as a function of temperature from 200 to 280° C. Details of the determination of the mass of bleed components are found in Reference 70. The results of the bleed tests indicated that SE-30 was more stable towards catalytic decomposition than OV-101. The most important conclusion is that glass capillary columns made from alkali glass exhibit much higher levels of bleed than borosilicate glasses, regardless of pretreatment and deactivation procedures.

Treatment of the alkali glass columns with gaseous hydrogen chloride and hydrogen fluoride converts the alkali and alkaline earth metal oxides (K_2O, Na_2O, BaO, CaO) into the corresponding halides which remain on the glass whereas on pretreatment with hydrochloric acid, the oxides are also converted to the halides but are removed from the columns during the washing step. It was noted[70] that a decrease in the sodium oxide level decreased the catalytic decomposition of methyl silicones. Methyl silicones coated onto the unpretreated borosilicate columns showed very low levels of bleed, although the borosilicate glass contains a small percentage (about 4%) of alkali metal oxides. However, alkali and barium carbonate, coated borosilicate columns exhibited strong bleed, and this suggests that the influence of the alkali metal oxides depends on the manner in which the oxides are chemically incorporated into the glass structure. One difficulty with the borosilicate capillary columns is the adhesion of the stationary phase film to the glass. The deposition of a layer of barium carbonate, silica, or sodium chloride greatly enhances the ability of the stationary phase to cover the glass capillary surface completely to minimize the effect of the active glass surface on column bleed. In terms of thermal stability of the stationary phase, the borosilicate columns, with or without the deposition of a prelayer, were considered a better choice than soda-lime columns.

In the following article, Schomburg et al.[72] extended their studies to consider the effect of various deactivation procedures on the extent of tailing. The polarity mixture which was studied included C_{10}, C_{11}, and C_{12} paraffins; C_{10}, C_{11}, and C_{12} esters; n-octanol; 2,6-dimethylphenol; 2,6-dimethylaniline; and dicyclohexylamine. Soda-lime glass capillary columns were used for the experiments. Simple baking of the methylposysiloxane stationary phase OV-101 at 350° C for 5 hr on an untreated soda-lime glass surface resulted in fairly good deactivation, i.e., minimal peak tailing up to about 220° C; however, the thermal stability of the stationary phase was poor beyond this point. When the baking treatment was preceded by a dealkalinization step with hydrogen chloride gas, the thermal stability of the OV-101 was improved, but tailing of the various solutes became severe. The optimum deactivation procedure involved dealkalinization followed by passage of a dilute solution of hydrogen fluoride gas in nitrogen (0.1 vol. %), followed by a 30-min baking at 450° C. This deactivation procedure was also found to work well for an alkylpolysiloxane stationary phase containing carborane groups, e.g., Dexsil® 400. Excellent peak symmetry was achieved for solutes containing NH-groups or more than one heteroatom.

The effect on surface activity resulting from the deposition of Carbowax® 20M in glass

capillary columns was investigated by Blomberg and Wannman.[73] For this series of tests Pyrex® and AR-glass (a soda-lime glass) tubing was cleaned in chromic acid-sulfuric acid, rinsed with water and acetone, dried, and drawn into 20 m × 0.6 mm O.D. capillaries. Some of the capillaries were etched with gaseous hydrogen fluoride and others with hydrochloric acid. After pretreatment, the capillaries were coated dynamically (at 20 mm/sec) with a solution of Carbowax® 20M (5% w/v) in dry dichloromethane. After coating, the solvent was evaporated. The ends of the capillary were sealed; some of the capillaries were baked for 24 hr at 280° C and then extracted with 16 mℓ of dichloromethane. A final coating of either Carbowax® 20M or a methylsilicone stationary phase, SP-2100 was placed on the capillary.

All the capillaries showed some degree of deactivation as a result of simple rinsing with dilute Carbowax® 20M solution. The hydrogen fluoride-etched Pyrex® and hydrochloric acid-etched AR-glass capillaries showed some residual activity towards alcohols, e.g., 1-octanol was not eluted from either column. However, AR-glass etched with hydrogen fluoride exhibited a lesser degree of activity in that the eluted 1-octanol exhibited severe tailing. Heat treatment significantly improved the degree of deactivation of all the Carbowax 20M treated capillaries; 1-octanol, dodecane, and naphthalene were eluted and gave symmetrical nontailing peaks.

Blomberg and Wannman[73] showed that the etching treatments were necessary to improve the wettability of the glass surface with Carbowax®. Coating of nonetched glass capillaries with Carbowax® resulted in the formation of lenses of thicker film on the capillary. However, after baking and extraction of the unetched capillaries, the degree of deactivation was acceptable to the authors.[73] With Pyrex® capillaries, etching increased the degree of deactivation, whereas with AR-glass the deactivation was slightly impaired.

For capillaries preheated with a thin nonextractable Carbowax® layer, thermal stabilities were similar for the differently pretreated columns. When SP-2100 was coated onto these capillaries, the AR-glass columns showed a drastically increased rate of bleed, while the analogous Pyrex®-glass columns exhibited a bleed rate comparable to capillaries coated with only Carbowax®. The authors[73] suggested that the active AR-glass surface combines with a large fraction of the Carbowax® functional groups, and this yields an inactive Carbowax® surface to which the SP-2100 adheres poorly.

Dandeneau et al.[74] have recently reported on the use of fused silica capillary columns. The columns are fabricated from amorphous silica (SiO_2) of greater than 99.9% purity. "Fused silica" is made by fusing synthetic silica produced by the combustion of silane or silicon tetrachloride in oxygen. The material prepared in this manner is essentially pure silicon dioxide that contains metal oxide impurities in the ppm range. This synthetic material contains fewer metal oxide impurities than natural quartz.

Preliminary studies by Dandeneau's group indicated that the fused silica surface was well suited to derivatization with the whole array of silanizing agents. Surface modification via specific coupling agents or silylation to increase the operating of the columns is thus possible.

A "polarity mixture" consisting of straight chain alcohols, cyclic and aromatic amines, alkanes, and phenols was used to test column performance. An unheated 22 m × 0.20 mm fused silica column was deactivated with Carbowax® 20M and coated with SP-2100. For all the compounds present in 1 to 2 ng amounts, peak tailing was minimal with this column. Test mixtures containing primary amines, complex alcohols, and PAHs, also showed insignificant tailing. A mixture of underivatized volatile fatty acids was separated on a fused silica column deactivated with Carbowax® 20M and further coated with Carbowax® 20M, and showed no tailing. Since Dandaneau et al.[74] have shown that fused silica columns are not completely inactive, some deactivation is usually necessary to eliminate residual activity, mainly of a Lewis acid nature. Preliminary

studies by the authors[74] indicate that the temperature stability of these columns is good and low levels of column bleed are observed for an SP-2100 column at 280° C.

It is difficult to fabricate completely inactive capillary columns which also provide surfaces that are suitable for thorough stationary phase coating. A concentrated effort must be made to perfect deactivation procedures which will result in excellent stationary phase stability at temperatures of 300° C or above. Minimal solute peak tailing must also be achieved. The procedures should be equally applicable to borosilicate or alkali glasses. At the present time, fused silica capillary columns provide the most inactive surface available.

3. Separations of PAHs

We have already emphasized the importance of resolving the numerous structural isomers of the PAH fraction. State of the art capillary column GC is the best approach at this time, but not perfect. Research is being conducted in order to develop capillary columns capable of complete resolution of all PAH isomers. The resolution of PAHs can be improved by development of either: (1) more highly efficient columns, or (2) more highly selective stationary phases. Most research effort falls in the first category, although development of PAH selective liquid crystal stationary phases for capillaries is also in progress. High separation efficiency, column deactivation, and thermal stability of the stationary phase are all factors which must be considered.

A system for identifying the hundreds of peaks which may be found in a capillary chromatogram is necessary. A well-defined procedure for determining retention indices for PAHs can aid in identification of compounds in unknown mixtures and can be useful in interlaboratory data comparisons. The need for such comparisons is even more critical when a GC/MS/data system is unavailable for positive peak identifications or standard samples of individual PAHs cannot be obtained.

Lee et al.[75] have addressed the problem of establishing a set of retention indices for PAHs for temperature programmed capillary column GC. The Kovats index system which determines retention indices relative to a series of homologous n-hydrocarbons has shortcomings when applied to PAHs. Retention data are reproducible only with identical chromatographic columns, isothermal conditions, and operating parameters. Lee et al.[75] pointed out additional factors which affect the reproducibility of retention data. These include (1) batch-to-batch differences in the chemical composition of the stationary phase, (2) reactions and decomposition of the stationary phase, (3) the degree of deactivation of the column material, (4) the type of column material, e.g. alkali, borosilicate glass, or fused silica, and (5) the type of temperature program employed. More reproducible retention data is obtained when the sample is chemically similar to the series of standard reference compounds. The need for a chemically homologous series to serve as reference compounds becomes even more critical when temperature programming is used since the retention time depends on temperature. In temperature-programmed GC, the approximate expression relating the retention index to retention time is:

$$I = 100\,\frac{T_{R\ (Substance)} - T_{R(Cz)}}{T_{R\ (C\,z+1)} - T_{R(Cz)}} + 100Z \qquad (5)$$

where $T_{R\ (Substance)}$ is the retention time of the compound for which the retention index is to be determined, $T_{R(C_z)}$ and $T_{R(C_{z+1})}$ are the retention times for the reference standards (usually n-alkanes) which bracket the compound of interest, and Z is the number of carbon atoms in the n-alkane standard that elutes just prior to the compound of interest.

For PAH analysis, a new set of standards (naphthalene, phenanthrene, chrysene, and

picene) was chosen instead of the n-alkanes. Retention indices for over 200 compounds were measured. The actual indices were determined by analyzing the PAHs with the four standard reference compounds on SE-52 coated glass capillary columns. SE-52 was selected because capillary columns coated with this stationary phase are highly efficient.[75] The columns were drawn from Kimble® R6 soda lime glass capillary tubing and were treated according to the following procedure: hydrochloric acid gas phase etch, formic acid wash, HMDS/TMCS gas phase silation, and static coating. A 12 m × 0.30 mm I.D. capillary column with a film thickness of 0.34 μm and a 12m × 0.28 mm I.D. capillary with a 0.17 μm film thickness were found to be equally efficient in terms of separation efficiency, thermal stability, and inertness. The oven temperature was programmed from 50 to 250° C at a rate of 2° C/min for each run.

The indices were calculated from Equation 5 in the same manner as when n-alkane standards are employed; however, in this case the Z stands for the number of rings in the PAH standard that elutes prior to the solute of interest. A necessary requirement is that all four standards be eluted during the temperature programming period.

The film thickness does not affect the value of the retention indices. Excellent reproducibility of the values of the PAH retention indices was obtained over a period of several months of repeated column use. The retention indices for over 200 compounds are listed in Table 24. To test the reliability of the retention indices as a function of different temperature programming rates, ten chromatographic runs were made. Three runs were at 4° C/min, and seven runs were at 2° C/min. The average 95% confidence limits for the series of PAH retention indices calculated from these runs were ±0.16 index units.

The development of this retention index system is relatively recent and has not yet been tested by other laboratories. The importance of standardization of retention data for complex mixtures of PAHs is obvious. We think that present research in the field of PAH separations with capillary-column GC will eventually lead to the universal adoption of such a system. However, at present, various researchers have individual preferences for different stationary phases, stationary phase concentrations, column materials, column lengths, deactivation procedures, etc. Until the optimum parameters have been established, little progress in the development of a standardized retention index system can be made.

The actual separations of PAHs and chromatographic results obtained by Lee et al.[75] can be assessed by inspection of Figure 34 and Table 24. The efficiency of the capillary columns was approximately 3000 plates per meter, and the peak width at half height averaged over all of the PAHs was 0.80 index units. Two PAHs differing by one retention index unit were stated to have better than 50% area resolution. The separation of the majority of PAHs shown in Figure 33 was good, although certain isomers remained unresolved, e.g., chrysene/triphenylene, the dibenzanthracenes, and also many of the alkyl isomers listed in Table 24. It can be seen (Figure 33), that some of the early eluting solutes exhibit peak tailing.

Other experimental results obtained by capillary column analyses of PAHs should be examined relative to the work of Lee et al.[75] in an effort to find improvements in: (1) column efficiency, (2) stationary phase selectivity towards PAHs, (3) stationary phase thermal stability, and (4) reproducibility of retention data. In addition, parameters which improve peak symmetry and decrease tailing must be determined and optimized.

Bjørseth et al.[76-79] have analyzed numerous environmental samples for PAHs using capillary column GC. Bjørseth considered SE-54 to be a better stationary phase than OV-1, OV-101, and OV-61 on the basis of separation efficiency, column bleeding, and long-term stability. The chromatographic conditions for analysis of PAHs in various environmental extracts were as follows: glass capillary column, 50 m × 0.34 mm I.D.

Table 24
PAH RETENTION INDICES

Compound number	Compound name	Average index	Standard deviation
1	1,2-Dihydronaphthalene	197.01	0.07[c]
2	1,4-Dihydronaphthalene	197.01	0.07[c]
3	Tetralin	197.04	0.05[d]
4	Naphthalene	200.00	
5	Benzo(b)thiophene	201.47	0.05[f]
6	Indoline	204.74	0.04[e]
7	Indole	205.26	0.08[d]
8	Quinoline	209.70	—[b]
9	Isoquinoline	215.61	1.01[d]
10	2-Methylnaphthalene	218.14	0.28[h]
11	2-Methylbenzo(b)thiophene	218.74	0.04[e]
12	Azulene	219.95	0.24[e]
13	Quinoxaline	220.37	0.05[d]
14	3-Methylbenzo(b)thiophene	221.02	0.03[e]
15	1-Methylnaphthalene	221.04	0.25[e]
16	8-Methylquinoline	223.02	0.06[e]
17	1,2,3,4-Tetrahydroquinoline	225.97	0.10[e]
18	6-Methylquinoline	229.82	0.03[e]
19	1,2,2a,3,4,5-Hexahydroacenaphthylene	232.70	—[b]
20	Biphenyl	233.96	0.24[d]
21	2-Ethylnaphthalene	236.08	0.16[e]
22	1-Ethylnaphthalene	236.59	0.14[e]
23	3-Methylindole	236.66	—[b]
24	2-Methylindole	237.42	0.21[f]
25	2,6-Dimethylnaphthalene	237.58	0.17[f]
26	2,7-Dimethylnaphthalene	237.71	0.07[d]
27	5-Ethylbenzo(b)thiophene	238.46	0.37[d]
28	2-Methylbiphenyl	238.77	0.04[e]
29	1,3-Dimethylnaphthalene	240.25	0.16[e]
30	1,4-Naphthoquinone	240.82	0.04[e]
31	1,7-Dimethylnaphthalene	240.66	0.25[e]
32	1,6-Dimethylnaphthalene	240.72	0.09[d]
33	2,2′-Dimethylbiphenyl	241.94	—[b]
34	2,6-Dimethylquinoline	242.43	0.29[e]
35	2,3-Dimethylnaphthalene	243.55	0.19[e]
36	1,4-Dimethylnaphthalene	243.57	0.16[f]
37	1,5-Dimethylnaphthalene	244.98	0.16[e]
38	Diphenylmethane	243.35	0.11[f]
39	Acenaphthylene	244.63	0.19[d]
40	2,2′-Bipyridyl	245.48	0.27[e]
41	1,2-Dimethylnaphthalene	246.49	0.30[e]
42	1,8-Dimethylnaphthalene	249.52	—[b]
43	2-Ethylbiphenyl	250.85	—[b]
44	Acenaphthene	251.29	0.14[g]
45	4-Methylbiphenyl	254.71	0.17[d]
46	3-Methylbiphenyl	254.81	0.15[f]
47	2,3-Dimethylindole	255.48	—
48	Dibenzofuran	257.17	0.05[e]
49	2-Methyl-1,4-naphthoquinone	259.23	0.11[e]
50	2,3,6-Trimethylnaphthalene	263.31	0.12[e]
51	1-Methylacenaphthylene	265.24	0.02[d]
52	2,3,5-Trimethylnaphthalene	265.90	0.14[e]
53	Dibenzo-*p*-dioxin	267.27	0.20[h]
54	Fluorene	268.17	0.15[g]
55	*trans*-1,2,3,4,4a,9a-Hexahydrodibenzothiophene	269.67	0.37[f]

Table 24 (continued)
PAH RETENTION INDICES

Compound number	Compound name	Average index	Standard deviation
56	*cis*-1,2,3,4,4a,9a-Hexahydrodibenzothiophene	271.39	0.27[f]
57	3,3'-Dimethylbiphenyl	271.87	—[b]
58	9-Methylfluorene	272.38	0.17[c]
59	2,3,5-Trimethylindole	272.57	—[b]
60	4,4'-Dimethylbiphenyl	274.59	—[b]
61	5H-Indeno(1,2-b)pyridine	279.31	0.19[c]
62	Xanthene	280.48	0.22[c]
63	9,10-Dihydroanthracene	284.89	0.19[c]
64	9-Ethylfluorene	284.99	0.20[c]
65	9,10-Dihydrophenanthrene	287.09	0.16[c]
66	1,2,3,4,5,6,7,8-Octahydroanthracene	287.69	0.20[c]
67	2-Methylfluorene	288.21	0.15[d]
68	1-Methylfluorene	289.03	0.04[d]
69	1,2,3,4,5,6,7,8-Octahydrophenanthrene	292.03	0.06[c]
70	1,2,3,4-Tetrahydrodibenzothiophene	294.30	0.12[c]
71	9-Fluorenone	294.79	0.26[d]
72	Dibenzothiophene	295.81	0.03[g]
73	1,2,3,4-Tetrahydrophenanthrene	297.21	—[b]
74	Phenanthrene	300.00	
75	Anthracene	301.69	0.08[g]
76	Benzo(h)quinoline	302.22	0.11[d]
77	9,10-Dihydroacridine	304.33	—[b]
78	Acridine	304.50	0.05[c]
79	1,2,3,4-Tetrahydrocarbazole	306.76	0.19[d]
80	Phenanthridine	308.79	0.23[a]
81	Benzo(f)quinoline	309.25	0.19[d]
82	Carbazole	312.13	—[b]
83	9-Ethylcarbazole	313.97	0.13[d]
84	1-Phenylnaphthalene	315.19	0.05[c]
85	1,2,3,10b-Tetrahydrofluoranthene	316.37	0.14[d]
86	9-*n*-Propylfluorene	318.01	0.20[c]
87	3-Methylphenanthrene	319.46	0.12[c]
88	2-Methylphenanthrene	320.17	0.12[d]
89	3-Methylbenzo(f)quinoline	320.77	0.24[d]
90	2-Methylanthracene	321.57	0.12[c]
91	*o*-Terphenyl	321.99	0.16[d]
92	4H-Cyclopenta(def)phenanthrene	322.08	0.15[d]
93	9-Methylphenanthrene	323.06	0.24[d]
94	4-Methylphenanthrene	323.17	—[b]
95	1-Methylanthracene	323.33	—[b]
96	1-Methylphenanthrene	323.90	0.08[c]
97	2-Methylacridine	324.46	0.05[c]
98	9-*n*-Butylfluorene	328.99	0.37[d]
99	9-Methylanthracene	329.13	0.19[d]
100	4,5,9,10-Tetrahydropyrene	329.69	0.24[c]
101	4,5-Dihydropyrene	330.01	0.02[d]
102	Thianthrene	330.13	0.26[c]
103	Anthrone	330.53	0.67[c]
104	2-Phenylnaphthalene	332.59	0.14[c]
105	9-Ethylphenanthrene	337.05	0.08[d]
106	2-Ethylphenanthrene	337.50	—[b]
107	3,6-Dimethylphenanthrene	337.83	0.14[d]
108	2,7-Dimethylphenanthrene	339.23	0.16[d]
109	1,2,3,6,7,8-Hexahydropyrene	339.38	0.17[c]
110	6-Phenylquinoline	342.45	0.40[c]

Table 24 (continued)
PAH RETENTION INDICES

Compound number	Compound name	Average index	Standard deviation
111	Fluoranthene	344.01	0.16[g]
112	9-Isopropylphenanthrene	345.78	0.28[c]
113	1,8-Dimethylphenanthrene	346.26	0.27[c]
114	2-Phenylindone	347.47	0.07[c]
115	Indeno(1,2,3-ij)isoquinoline	347.57	0.19[c]
116	9-*n*-Hexylfluorene	348.54	0.10[c]
117	9-*n*-Propylphenanthrene	350.30	0.17[c]
118	Pyrene	351.22	0.08[d]
119	9,10-Dimethylanthracene	355.49	0.03[c]
120	Benzo(1mn)phenanthridine	358.53	0.28[c]
121	9-Methyl-10-ethylphenanthrene	359.91	0.19[c]
122	*m*-Terphenyl	360.73	0.03[c]
123	Benzo(kl)xanthrene	361.38	0.15[c]
124	4H-Benzo(def)carbazole	364.22	0.12[d]
125	*p*-Terphenyl	366.10	0.16[g]
126	Benzo(a)fluorene	366.74	0.13[g]
127	11-Methylbenzo(a)fluorene	367.04	0.14[d]
128	9,10-Diethylphenanthrene	367.97	0.15[d]
129	1-Methyl-7-isopropylphenanthrene	368.67	0.19[c]
130	Benzo(b)fluorene	369.39	0.15[c]
131	4-Methylpyrene	369.54	0.13[c]
132	2-Methylpyrene	370.15	0.44[d]
133	4,5,6-Trihydrobenz(de)anthracene	370.86	—[b]
134	1-Methylpyrene	373.55	0.11[c]
135	3,5-Diphenylpyridine	373.79	0.18[c]
136	5,12-Dihydronaphthacene	381.56	—[b]
137	9,10-Dimethyl-3-ethylphenanthrene	381.85	0.18[c]
138	9-Phenylcarbazole	382.09	—[b]
139	1-Ethylpyrene	385.35	—[b]
140	2,7-Dimethylpyrene	386.34	0.06[d]
141	1,2,3,4,5,6,7,8,9,10,11,12-Dodecahydrotriphenylene	386.36	0.29[c]
142	11-Benzo(a)fluorenone	386.41	0.06[d]
143	1,1'-Binaphthyl	388.38	0.23[d]
144	Benzo(b)naphtho(2,1-d)thiophene	389.26	0.16[c]
145	Benzo(ghi)fluoranthene	389.60	0.06[d]
146	Benz(c)phenanthrene	391.39	0.40[c]
147	Benz(c)acridine	392.50	0.13[c]
148	9-Phenylanthracene	396.38	—[b]
149	Cyclopenta(cd)pyrene	396.54	0.08[d]
150	Benz(a)anthracene	398.50	0.08[c]
151	Benz(a)acridine	398.74	—[b]
152	Chrysene	400.00	
153	Triphenylene	400.00	0.01[c]
154	Benzo(a)carbazole	401.82	—[b]
155	1,2'-Binaphthyl	405.38	0.05[c]
156	7-Benz(de)anthrene	406.54	0.34[d]
157	9-Phenylphenanthrene	406.90	0.14[c]
158	Naphthacene	408.30	0.22[d]
159	Benzo(b)carbazole	410.12	—[b]
160	11-Methylbenz(a)anthracene	412.72	—[b]
161	2-Methylbenz(a)anthracene	413.78	0.44[c]
162	1-Methylbenz(a)anthracene	414.37	0.17[c]
163	1-*n*-Butylpyrene	414.87	0.12[d]
164	1-Methyltriphenylene	416.32	—[b]
165	9-Methylbenz(a)anthracene	416.50	0.20[d]

Table 24 (continued)
PAH RETENTION INDICES

Compound number	Compound name	Average index	Standard deviation
166	3-Methylbenz(a)anthracene	416.32	—[b]
167	9-Methyl-10-phenylphenanthrene	417.16	—[b]
168	8-Methylbenz(a)anthracene	417.56	0.04[c]
169	6-Methylbenz(a)anthracene	417.57	0.30[c]
170	3-Methylchrysene	418.10	0.17[d]
171	5-Methylbenz(a)anthracene	418.72	—[b]
172	2-Methylchrysene	418.80	—[b]
173	12-Methylbenz(a)anthracene	419.39	0.45[d]
174	4-Methylbenz(a)anthracene	419.67	—[b]
175	5-Methylchrysene	419.68	—[b]
176	6-Methylchrysene	420.61	0.04[c]
177	4-Methylchrysene	420.83	0.16[c]
178	2,2′-Biquinoline	421.12	—[b]
179	1-Phenylphenanthrene	421.66	0.31[c]
180	1-Methylchrysene	422.87	0.06[c]
181	7-Methylbenz(a)anthracene	423.14	—[b]
182	*o*-Quaterphenyl	423.63	—[a]
183	2,2′-Binaphthyl	423.91	—[b]
184	2,(2′-Naphthyl)-benzo(b)thiophene	428.11	0.37[d]
185	1,3-Dimethyltriphenylene	432.32	—[b]
186	1,12-Dimethylbenz(a)anthracene	436.82	—[b]
187	Benzo(j)fluoranthene	440.92	—[b]
188	Benzo(b)fluoranthene	441.74	0.48[c]
189	Benzo(k)fluoranthene	442.56	—[b]
190	7,12-Dimethylbenz(a)anthracene	443.38	0.12[c]
191	1,6,11-Trimethyltriphenylene	446.24	—[b]
192	Dinaphtho(1,2-b; 1′,2′-d)furan	450.20	—[b]
193	Benzo(e)pyrene	450.73	0.17[c]
194	Dibenzo(c,kl)xanthene	451.57	—[b]
195	Benzo(a)pyrene	453.44	—[b]
196	Perylene	456.22	0.29[c]
197	1,3,6,11-Tetramethyltriphenylene	461.72	—[b]
198	3-Methylcholanthrene	468.44	—[b]
199	*m*-Quaterphenyl	472.81	0.23[c]
200	Indeno(1,2,3-cd)pyrene	481.87	0.09[c]
201	Pentacene	486.81	—[b]
202	*p*-Quaterphenyl	488.18	0.38[d]
203	Dibenz(a,c)anthracene	495.01	0.08[b]
204	Dibenz(a,h)anthracene	495.45	—[a]
205	Benzo(b)chrysene	497.66	—[a]
206	Picene	500.00	
207	Benzo(ghi)perylene	501.32	0.18[c]
208	Dibenzo(def,mno)chrysene	503.89	—[b]
209	2,3-Dihydrodibenzo(def,mno)chrysene	503.91	—[b]

[a] One determination.
[b] Two determinations.
[c] Three determinations.
[d] Four determinations.
[e] Five determinations.
[f] Six determinations.
[g] Seven determinations.
[h] Eight determinations.

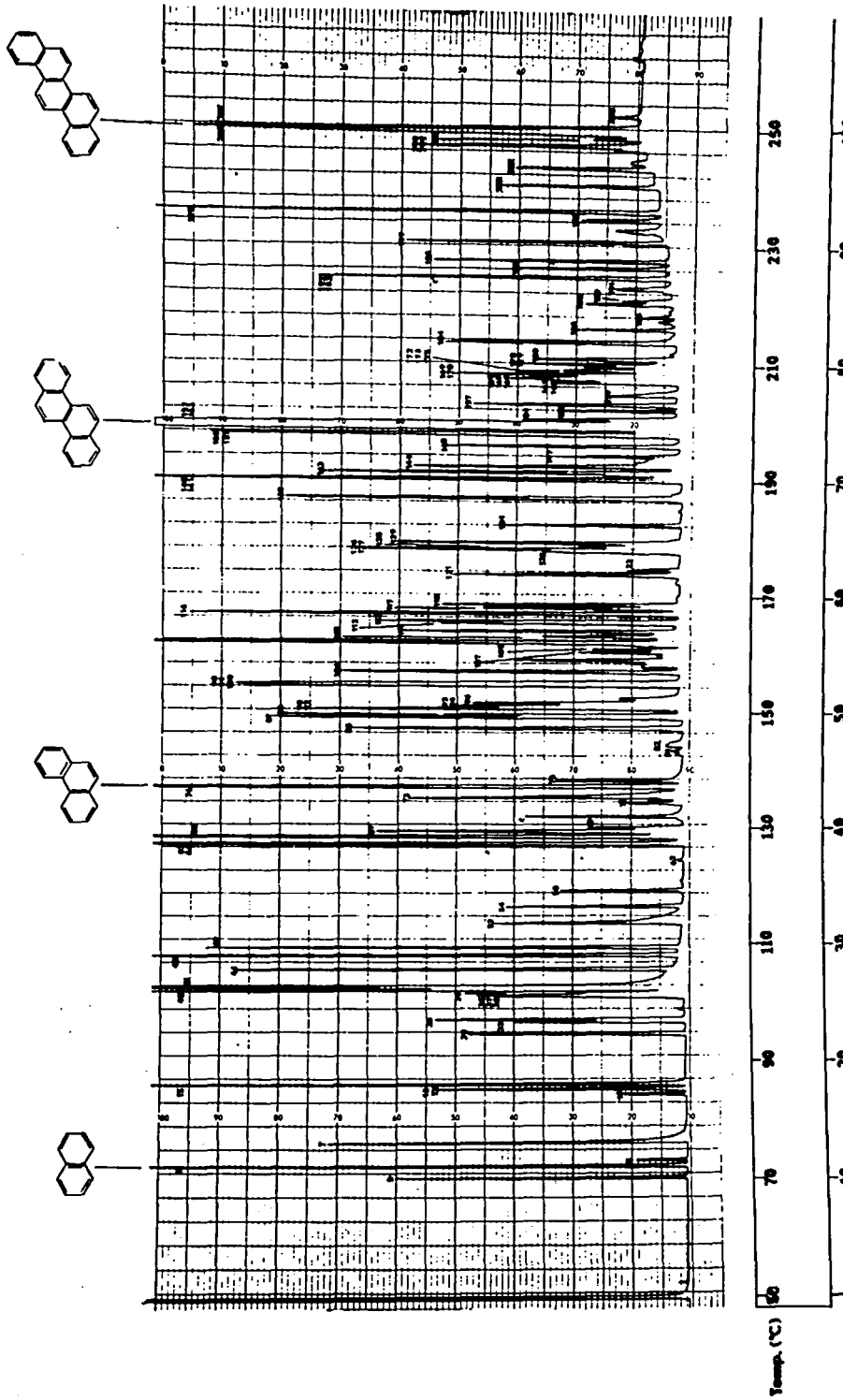


FIGURE 34. Capillary column gas chromatogram of standard PAHs on a 12 m × 0.29 mm, I.D., glass capillary coated with a 0.34 μm film of SE-52. Numbered peaks refer to compounds listed in Table 24.

coated with SE-54; hydrogen carrier gas at about 3 mℓ/min at ambient temperature; injector and detector temperatures, 275° C; initial column temperature, 115° C; programmed temperature rate, 3° C/min; final temperature, 260° C. Since these results were reported in the literature, Bjørseth has moved to Battelle's Columbus Laboratories, and the deactivation procedure reported by Meuser et al.[69] (Section V. C.2) is most likely the preferred procedure. The separations which were obtained on an extract from marine sediments containing PAHs are illustrated in Figure 35. The isomers: anthracene/phenanthrene, chrysene/B(a)A, and B(e)P/B(a)P were resolved. However, the same difficulties that were encountered by Lee et al.[75] were observed in this work. Chrysene and triphenylene were not resolved and the benzofluoranthenes, as well as many of the methylated PAHs, were only partially resolved.

The capillary column GC parameters for analyses of environmental samples for PAHs, as reported by Meuser et al.[69] at the recent symposium on PAHs, were as follows: 15 m × 0.28 mm glass capillary coated with SE-52, oven temperature, 100 to 320° C at a rate of 3° C/min, carrier gas, hydrogen at 50 cm/sec. Although no chromatograms are yet available for publication, it was reported at the symposium that coronene could be detected within 45 min. Investigations to apply these techniques to environmental samples are currently in progress.

Lao and Thomas[80] have not been able to report any significant improvements in separation efficiency towards PAHs using WCOT glass capillary columns with Dexsil® 300 and SE-52. Once again, the benzofluoranthenes and chrysene and triphenylene were unresolved.

Blomberg et al.[81-83] have attempted to solve the problem of poor stationary phase stability by synthesizing chemically bonded phenyl polysiloxanes *in situ* in the glass capillary column. The resulting stability of these stationary phases was explained in terms of a slight degree of cross-linking in the stationary phase and chemical bonding of the polysiloxane to the glass surface. With these columns, slightly improved separations of PAHs including the partial separation of chrysene from triphenylene were reported and good thermal stability (low column bleed) of the columns up to 310° C was noted. The lifetime stability of soda lime glass capillary columns of this type were reported to be approximately 3 months when performing ten analyses per day.

At the present, it appears that the various methyl and phenyl silicone stationary phases lack the selectivity towards PAHs necessary to resolve the various isomers. The development of more highly efficient capillary columns does not obviate the search for more selective stationary phases. Perhaps greater emphasis should be placed upon the application of liquid crystal stationary phases which are known to possess remarkable selectivity towards isomeric PAHs, to capillary columns. No reports of such applications can be found in the literature.

Greater progress has been made towards the fabrication of capillary columns with enhanced thermal stability at temperatures up to 350° C. The introduction of fused silica capillary columns has led to the successful analysis of the EPA base-neutral extractables using a maximum column temperature of 350° C.[83a] Figure 36 illustrates the low bleed level of the SE-54 capillary column at 350° C. Recently, Maskarinec and Olerich[84] reported on preliminary studies which indicate that Dexsil® 400 coated glass capillary columns are thermally stable up to 350° C. They indicated that these capillaries should be useful for analyses of high molecular weight PAHs.

D. Methods of Detection

The capillary or packed column used for the separation must be coupled to a suitable detector. Four detector systems which have been applied to the determination of PAHs are (1) flame ionization, (2) gas phase fluorescence, (3) photoionization, (4) mass

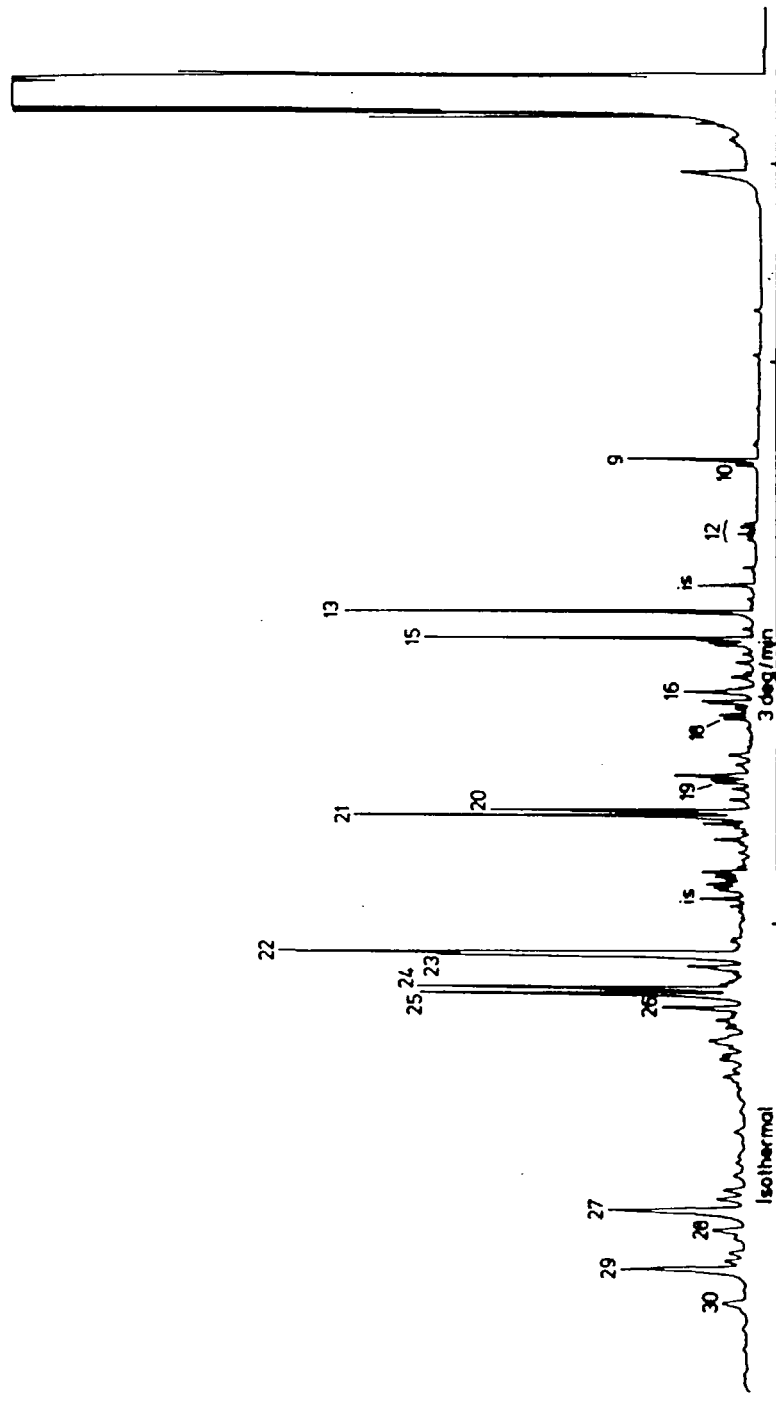


FIGURE 35. Glass capillary gas chromatogram of PAH in marine sediments outside an electrochemical plant. The stationary phase is SE-5 Numbers above the peaks refer to compounds listed in the figure key. Peak legend: (9) phenanthrene, (10) anthracene, (12) methylphenanthrenes an anthracenes, (13) fluoranthene, (15) pyrene, (16) benzo(a)fluorene, (18) 1-methylpyrene, (19) benzo(c)phenanthrene, (20) benz(a)anthracene, (2 chrysene/triphenylene, (22) benzo(b)fluoranthene, (23) benzo(k)fluoranthene, (24) benzo(e)pyrene, (25)benzo(a)pyrene, (26)perylene, (27)indeno(1,2,: cd)pyrene, (28) dibenz(a,h)anthracene, (29) benzo(ghi)perylene, (30) anthanthrene.

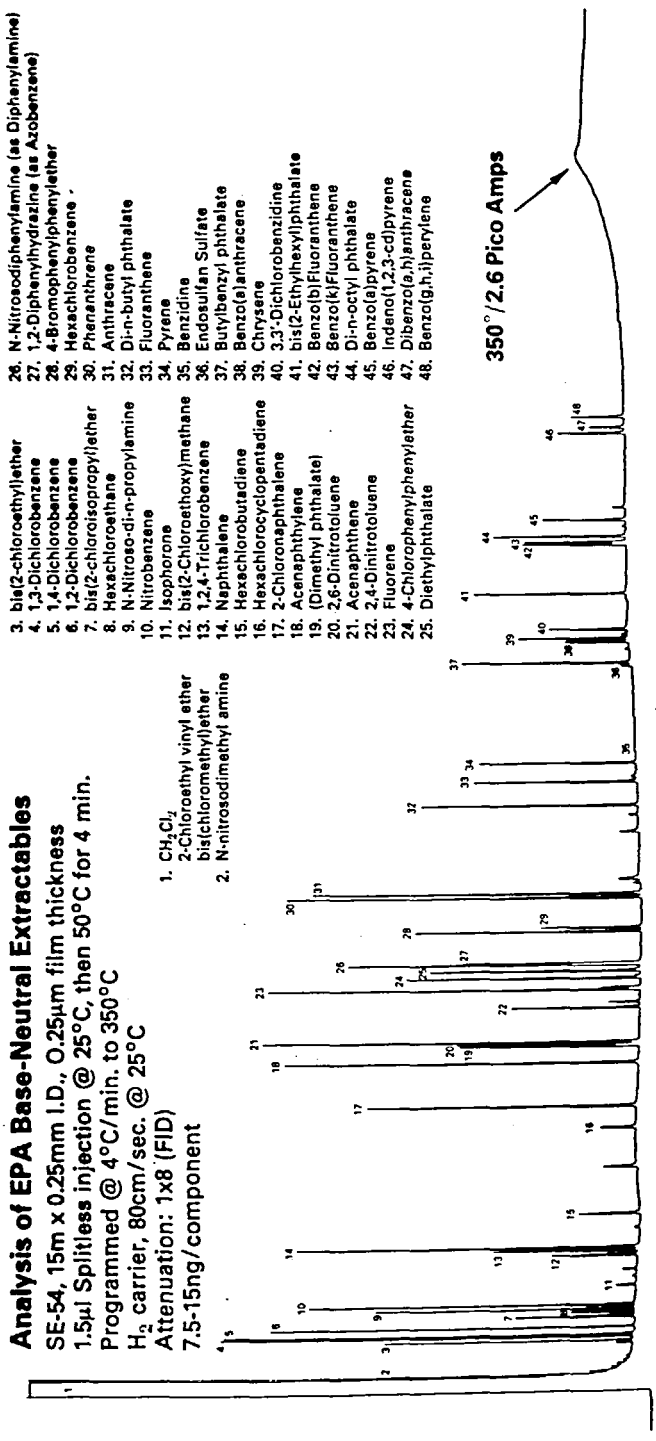


FIGURE 36. Illustration of the bleed level at 350°C resulting from an analysis of EPA Base-Neutral Extractables using a fused silica glass capillary.

spectrometry. Since GC/MS analysis is a separate technique, it will be discussed in a separate section.

The flame ionization detector (FID) responds with high sensitivity to essentially all organics and is the most commonly used detector. The design and characteristics of a flame ionization detector are discussed by Grob.[85]

For the determination of PAHs, two characteristics of the FID are important: (1) sensitivity to low levels of PAHs, and (2) selectivity towards PAHs. In practice, the FID meets the first requirement. The second cannot be achieved since the FID responds to all organics and not just PAHs.

Quantitative analytical results from flame ionization detection depend on analysis of the chromatographic peaks, and for this purpose electronic integration is most commonly employed[86,87] because it is more accurate and precise than other methods.[85] For the quantitation of the chromatographic peaks, the area of the peak is compared to the peak area of an internal standard,[86,88] and 1-chlorotetradecane[86] and octacosane[88] are commonly used internal standards for this purpose. Quantitative PAH results are obtained by using a linear relationship between peak areas (PAH/internal standard) and the concentration ratio (PAH sample/internal standard).

Limits of detection are defined in many different ways by various authors; thus they can only be used as an order of magnitude figure to compare detector capabilities. The treatment of limits of detection for HPLC by Christensen and May (Section IV.C) is also valid for GC. Acheson et al.[87] were able to detect 10 ng amounts of most PAHs including B(*a*)P and chrysene with a FID. Basu and Saxena[5] defined detection limits as a minimum output response of five times the background noise level and a maximum sample size of 5 $\mu\ell$. They were able to detect as little as 11.9 ng of B(*a*)P, 10.1 ng of the benzofluoranthenes, and 14.7 ng of IP. Burchill et al.[88] found the effective limit of detection for B(*a*)P to be 1.6 ng with a maximum of 1.0 $\mu\ell$ of sample. This corresponds to a peak height of 5 mm or a peak area of 100 mm at a 5 mm/min chart drive and at the lowest usable attenuation. Radecki et al.[89] reported 30 ng to be the minimum detectable amount of B(*a*)P using a FID. In our own studies of detection limits for PAHs with a FID, detection limit was defined as a minimum peak height of two times the noise level. We were able to detect 0.1 to 10 ng amounts for individual PAHs from naphthalene to coronene. As a general rule, 0.1 to 30 ng amounts of PAHs are detectable with a FID.

Although fluorescence detectors are commonly used with HPLC systems for determinations of PAHs, they have not been widely used with GC. The main reason is that the GC effluent is a gas, whereas in HPLC it is a liquid. However, some studies using fluorescence detection with a gas chromatograph have been carried out.

Freed and Faulkner[90] coupled a modified Aminco-Bowman® spectrophotofluorimeter to a GC by connecting the GC to a heated fused silica flow cell (of 1.5 mℓ volume) with a heated transfer line. Fixed slits were placed into the cell holder to select various bandpasses. The cell configuration is shown in Figure 37. The spectrophotofluorometer was modified to enable the spectrum from 200 to 800 nm to be scanned repetitively at 5-sec intervals and an internal standard was used to minimize light source variations on detector response. Calibration curves were linear over a range from 1 ng to 10 μg of PAH. At an excitation wavelength of 313 nm and an emission wavelength of 400 nm, 0.5 ng of pyrene was detectable when the detection limit was defined as a signal-to-noise ratio of two. Further instrumental details are found in the reference.

Robinson and Goodbread[91] devised a simple type of fluorescence detector for GC analyses of PAHs. With their method, the sample was excited over a broad wavelength range rather than a selected wavelength, and the resulting emission was measured over a wide bandwidth. In this manner, a gain in sensitivity was achieved at the cost of reduced selectivity. The cell system and instrumental schematic are shown in Figure 38. A

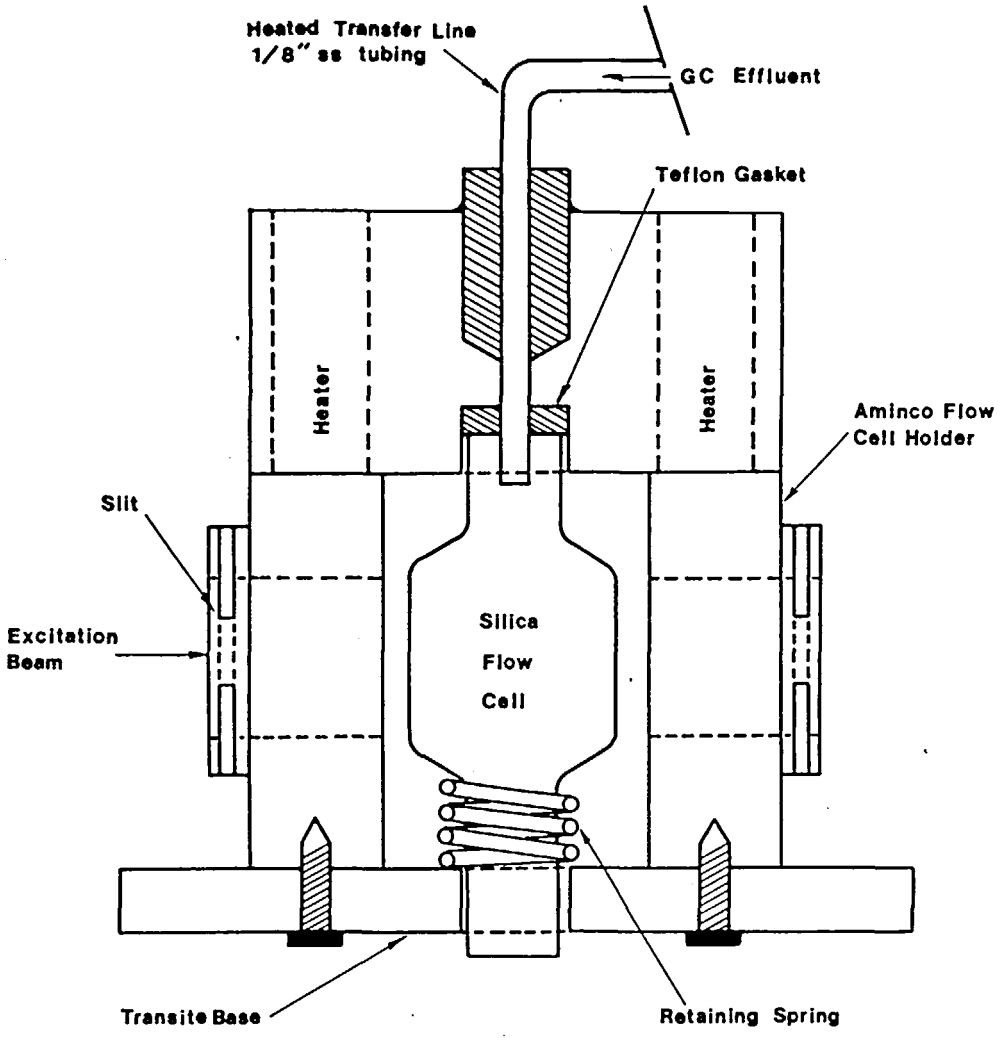


FIGURE 37. Schematic diagram of the heated fluorescence cell, as viewed from the emission monochromator. For clarity, the facing slit and the thermal insulation have not been shown.

cylindrical cell was used in an attempt to minimize the effect of stray light. Filters were used to limit the excitation (250 to 380 nm) and emission (400 to 500 nm) wavelengths. The 380 to 400 nm range served as a neutral zone to isolate interfering scattered radiation from the Xe-Hg light source. In this experiment the detector was not actually coupled to a GC, but rather simulated conditions were used in which the PAH sample was vaporized and introduced into the cell along with carrier gas. Stopped flow and moving stream procedures were investigated and it was found that the sensitivity was greater with the former technique. By correlating peak heights and sample concentrations, a value of 1.0 ng of anthracene was considered as a minimum detectable limit. The results are comparable with those obtained by Freed and Faulkner,[90] and the major advantage is that the apparatus for the wide band fluorescence method is somewhat less expensive.

Caddy and Meek[92] have also devised a simple fluorescence type of GC detector to be used simultaneously with the FID. A sample splitter was used and the portion that was directed to the fluorescence flow cell passed first to a refrigerated zone or cooler where

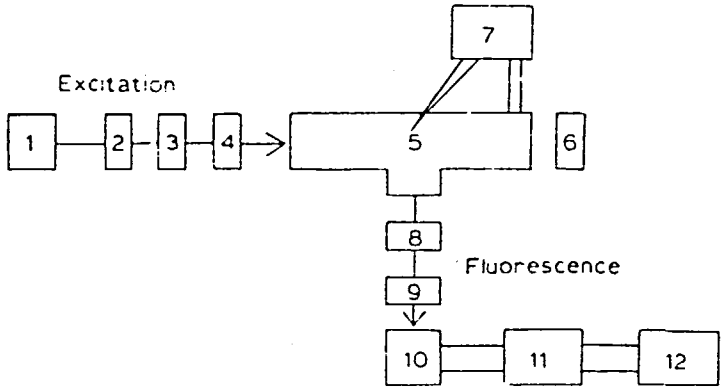


FIGURE 38. Schematic diagram of the equipment. (1) Xe-Hg lamp; (2) H_2O filter; (3) excitation filter, 250 to 380 nm; (4) lens; (5) cell; (6) mirror; (7) temperature control unit; (8) lens; (9) fluorescence filter, 400 to 500 nm; (10) P.M. tube; (11) P.M. tube power supply and amplifier; (12) X-Y recorder.

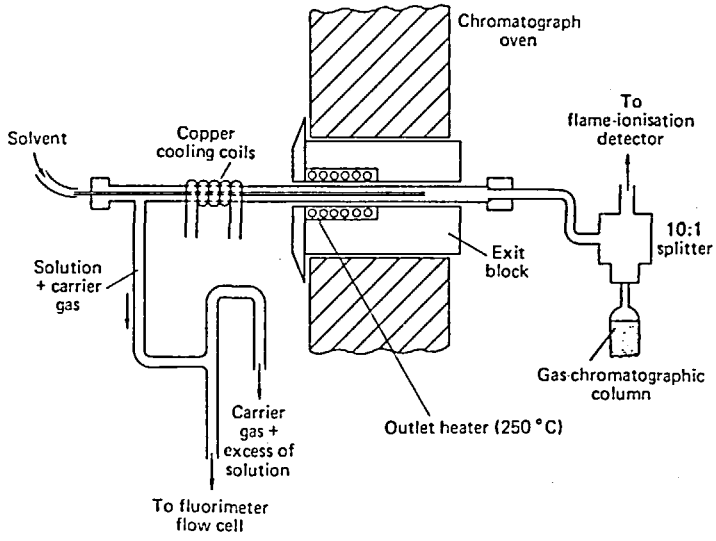


FIGURE 39. Gas chromatograph — fluorimeter interface.

solvent was pumped from an external source (see Figure 39). Some solvent and solute combined in the cooler, and the resulting solution along with carrier gas flowed through a tube to the fluorescence cell. Along the way, some solution and carrier gas were vented by means of the T-joint shown in Figure 39. Wide-band excitation (280 to 330 nm) and emission (350 to 450 nm) wavelength ranges were used. The simultaneous FID and fluorescence signals were displayed together. Peak area evaluation indicated linear response over the concentration range examined with a limit of detection of 10 ng for most PAHs. Oils and sewage sludge samples were analyzed by this method.

Cooney and Winefordner[93] have definitively analyzed the instrumental effects on detection limits for gas phase fluorescence detection. They examined in critical detail the [illegible] optical systems which affect the signal to noise ratio. The origins of limiting

noise were examined, and the major contribution was considered to be stray visible light at the emission wavelength which was scattered by the sample cell and subsequently measured by the detector. They suggested that detection limits on the order of tens of picograms could be achieved if some practical methods to improve system performance could be developed and implemented. They suggested the use of highly intense and stable light sources, such as a flash pumped dye laser with maximum output in the region of maximum PAH absorption, i.e., 200 to 300 nm. Further, they recommended the use of improved optical systems such as monochromators with holographically produced gratings to be used with the laser excitation. Implementation of these recommendations would involve the optimization of grating efficiencies in the UV region and a grating design to minimize stray light.

Until the developments suggested by Cooney and Winefordner are implemented, there is no sensitivity advantage in the use of fluorescence over flame ionization detection. At present, the FID is the most widely used GC detector for PAHs. However, gas phase fluorescence detectors have great potential since in HPLC/fluorescence systems, it has been possible to achieve detection limits for PAHs in the picogram range, as compared to the nanogram range for any of the present GC detectors.

The photoionization detector (PID) was developed in 1976 by Driscoll et al. at HNU Laboratories.[94-97] The application of a GC-PID system to analysis of alkanes, simple aromatics, PAHs, and many other organics has been reported. The sensitivity of the PID system is 10 to 50 times greater than that of the FID system.[98]

In photoionization, UV radiation of sufficient energy is absorbed by a molecule to cause ionization. An 11.0-eV lamp source is sealed into the detector. Radiation from this source will ionize molecules with an ionization potential less than 11.0 eV as they elute from the column. The ions formed are accelerated to a collecting electrode. The concentration of organic molecules can be directly related to the ion current collected.

With the present radiation sources that have been incorporated into this detector, compounds having ionization potentials less than about 11.0 eV can be detected by the PID method; these include aliphatic hydrocarbons (excluding methane), cyclic hydrocarbons, aromatics including PAHs, and other organics. If a lamp source with an energy of 10.0 eV is used, PID detection of many solvents, e.g., methanol and chloroform can be avoided. The ability to interchange sources adds a degree of selectivity and, in general, a lamp with an energy output just capable of ionizing the solutes of interest is selected.

Some advantages of the PID have been listed in a pamphlet by HNU.[99] These include (1) lower limits of detection for organics by a factor of 10 to 50 over the FID, (2) a linear dynamic range of operation over several orders of magnitude of concentration, from 2 pg to 30 μg, (3) the capability of operating at a detector temperature of 300° C and a column temperature of 280° C, (4) the capability of using the detector in series with other detectors, since the PID technique is virtually nondestructive towards the sample, (5) the easy adaptability of a PID to existing GCs, and (6) the relatively low cost, approximately $3500.

The advantages are important to analysts working with PAHs. Since the modification in PID hardware to allow higher operating temperatures is only a few years old, and because HNU Inc. is the only company presently marketing PIDs, there have been very few reports in the literature on PID applications to PAH determinations. Oyler et al.[98] have analyzed PAHs and their chlorinated derivatives in water and found the PID to be 10 to 40 times more sensitive to these compounds than the FID. Detection limits of 50 to 100 pg were reported for the PAHs studied: fluoranthene, anthracene, phenanthrene, and fluorene. They cited the lack of a signal for water or the acetonitrile solvent as a distinct advantage of the PID.

Driscoll et al.[100,101] have studied the relative response factors of PID and FID for lower

molecular weight hydrocarbons and pesticides. In all cases, the response of the PID was greater than the FID; this resulted in lower limits of detection when PID was used. The greatest increase in sensitivity was observed for aromatics and pesticides. The PID was 35 times more sensitive to aromatics than the FID, and 7 times more sensitive to alkanes such as n-hexane.

Hester and Meyer[102] have used the PID to detect low molecular weight aromatics in airborne particulate matter. At maximum sensitivity, with detection limits defined as an S/N ratio of two, benzene, toluene, and m-xylene could be detected at levels of less than 1 ppb.

The advantages of PID should lead to its increased application as a detector for GC. The introduction of the high-temperature PID should prove especially valuable for the determination of the higher molecular weight PAHs. A further advantage of the nondestructive feature of PID is that this detector can be used in series with a mass spectrometer, and we predict that reports of this application will appear shortly.

E. Gas Chromatographic Detection With a Mass Spectrometer

When the mass spectrometer is used as a detector for the GC, an output similar to that from a GC-FID system is obtained. The interfacing is a problem in that the effluent from the GC must be stripped of much of the carrier gas before being introduced into the mass spectrometer. A number of detailed discussions on how this can be accomplished are available.[103,104] Once the mass spectrometer and gas chromatograph are interfaced, the mass spectrometer records the mass spectra of the GC effluent as elution from the column proceeds, and these mass spectra are stored in a data system. All the ions in each spectrum are summed and recorded as a function of elution time or mass spectrum number. The plot of the total ion current vs. time or spectrum number gives a trace similar to that of a FID output. There may be some differences in intensity of response that depend on the difference in FID and mass spectrometer sensitivities to a particular compound. The retention times of the GC will not be affected if the system is properly designed.

The use of a mass spectrometer as a GC detector, though not as sensitive as some other detectors, provides a detail of data unmatched by other techniques. Figure 40 illustrates the different methods of data presentation which can be obtained with a GC/MS data system. Not only can the mass spectrum that is being scanned as well as the total ion chromatogram be presented in real time on a split screen as they are being generated, but many other types of ouput that are useful to the analyst can also be produced. Three-dimensional spectra involving mass, intensity, and time can be generated.

Because of the vast amounts of data produced by the mass spectrometer, most modern GC/MS systems are equipped with a dedicated computer to provide the output desired. The mass spectrometer scans the GC effluent at present intervals, e.g., every 5 sec, over the course of the elution of the sample, and the spectra are stored in the computer. At the end of the GC separation, several hundred spectra may be stored in the computer.

The computer can normalize peaks, subtract background column bleed, and plot the mass spectra — in addition to acquiring data. One form of computer-assisted data presentation is the generation of a mass chromatogram from the mass spectra stored in the data system. The intensity of a preselected ion in each spectrum stored is plotted as a function of spectrum number. These mass chromatograms are constructed from a characteristic ion mass; e.g., a mass chromatogram of m/e:252 would serve to identify the elution time of all $C_{20}H_{12}$ PAHs. The sensitivity of the mass chromatogram is less than that of the total ion chromatogram because only a fraction of the ions of each spectrum are plotted; however, the advantage of being able to select the peaks of the chromatogram that contribute to a particular mass overcomes this drawback. In the case of PAHs where the molecular ion is very intense, this loss in sensitivity is not serious.

In cases where the chemical composition(s) of the sample is(are) known in advance,

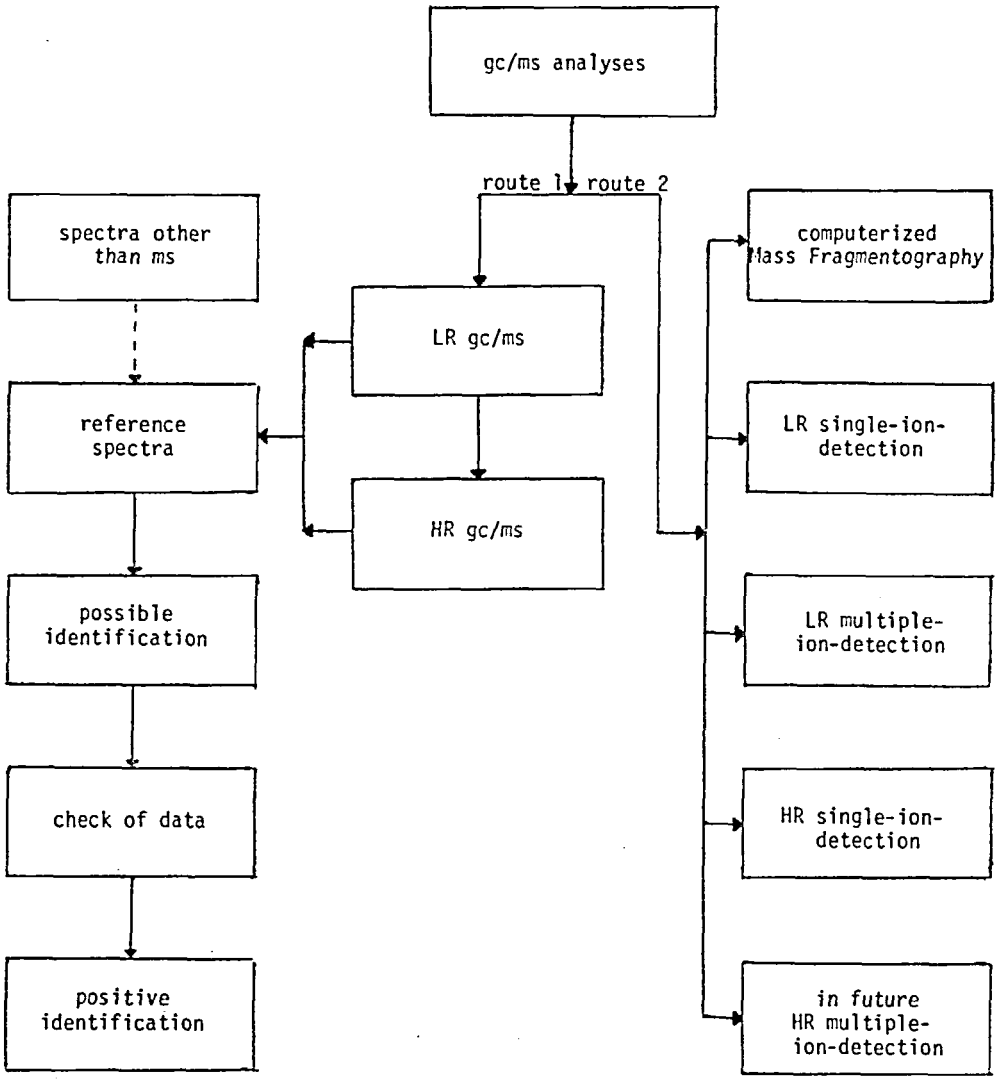


FIGURE 40. Flow chart of the different procedures applied in GC/MS analyses. Route 1, multicomponent analysis of complex mixtures; route 2, qualitative and quantitative trace analysis of complex mixtures.

another GC/MS technique, selected ion monitoring (SIM), where the intensity of a preselected characteristic ion is measured, can be used. Details on this method have been presented by Fenselau.[105] In SIM the ion intensities at a preselected m/e value are measured as the sample is eluted from the gc. By monitoring m/e:252 in a PAH analysis, the elution times of all the $C_{20}H_{12}$ PAH isomers can be determined. An increased level of sensitivity is achieved with SIM since the total scan time is dedicated to the collection of mass 252 ions rather than to measuring all the masses in the spectrum. This technique becomes multiple ion monitoring when it is expanded to include a number of characteristic ions which can be used to identify the components of a mixture.

Extensive reference libraries of mass spectra are available to assist in the interpretation of the large amount of data acquired in a GC/MS run. Several papers[106-108] have discussed the problems of data interpretation. McGuire et al.[108] discuss the approaches

Table 25
FRAGMENTATION PATTERNS OF SOME $C_{20}H_{12}$ ISOMERS

Relative intensities	Mass/charge ratio (m/e ratio)				
	252	126	253	250	125
B(a)P	100	23	21	16	15
B(e)P	100	21	23	25	21
Perylene	100	21	22	21	17
B(k)F	100	27	23	20	16
B(b)F	100	23	23	19	15
B(j)F	100	22	23	21	19

the Environmental Protection Agency have devised to simplify GC/MS analysis and subsequent data interpretation of organic pollutants in water.

Mass spectral interpretation of PAHs and their derivatives is relatively straightforward and has been discussed by Borwitzky and Schomburg.[71] Unsubstituted PAHs, as well as the monomethyl derivatives, exhibit very intense molecular ions, which are usually the base peaks. From the examination of the fragmentation patterns, a distinction between isomeric PAHs is generally quite difficult, if not impossible. The prominent peak intensities for the PAH isomers of MW:252 are listed in Table 25. It can be seen that the identification of the isomers must be made in conjunction with elution time data.

The mass spectra of the unsubstituted PAHs exhibit hydrogen cleavage of the molecular ion yielding M-1 and M-2 ions. M-26, M/2e, the doubly charged molecular ion, and (M-26)/2e ions are also observed. For PAHs with CH_2 linkages, e.g., fluorene and acenaphthene, the molecular ion remains the base peak followed by M-1, M-2, M-3, and M-4 as a result of hydrogen cleavage. The ions M-26 as well as M/2e and (M-26)/2e are also found.

Finnigan and Knight[109] have employed glass capillary GC/MS in analyses of Alaskan oil samples to determine the PAH content. Figure 41 shows the total ion chromatogram of the oil sample. A total of 1825 scans were taken over a period of 45 min. By searching all the recorded mass spectra for masses 178, 228, and 252, the scan number associated with PAHs of these molecular weight were identified.

The specific ion search is exemplified in Figure 42, where a total ion chromatogram overlapped with a specific ion search for m/e:178 is shown. Anthracene and phenanthrene give rise to almost identical mass spectra; therefore, standards or exact retention times are needed to verify which of the two isomers is being identified.

Benoit et al.[110] have identified a number of PAHs in Ottawa drinking water using GC/MS. The XAD-2 resin extract concentrate was injected onto a 3% OV-17 packed column for separation. The total ion chromatogram contained a number of weak and poorly defined peaks which made the task of obtaining identifiable mass spectra free of interfering ions difficult, even with the aid of a computer-generated background subtraction routine. To identify individual components, mass chromatograms were generated at selected ion currents by a computer search of the accumulated data for the ion of interest. The retention times for each PAH were determined by the analysis of a standard solution, and the data from the XAD-2 extract were searched for the particular ion within the appropriate retention time window. The molecular ion and the next most abundant ion were selected as the characteristic ions for each PAH. A particular PAH compound was considered to be identified if the two characteristic ions of the compound

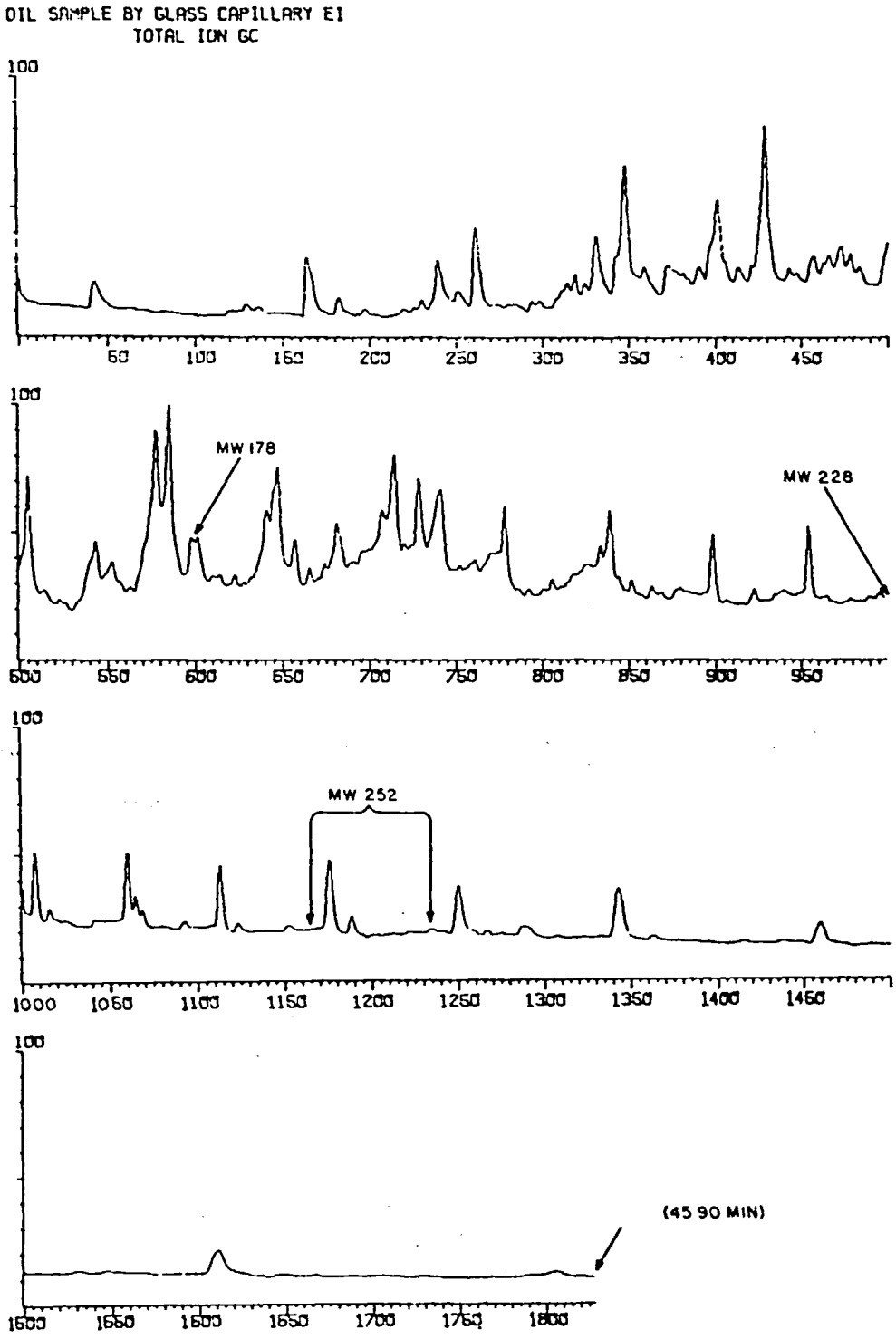


FIGURE 41. Total ion chromatogram of Alaskan oil sample. The m/e values marked on the chromatograms show where specific PAHs were found by limited mass search. Conditions: 20m × 0.02 in I.D. SE-30 glass capillary; column temperature, ambient to 250°C at 4°C/min; carrier gas, He at 2 mℓ/min flow rate; injector temperature, 250°C; sample size, 0.2 μℓ (splitless injection); mass range scanned, 40 to 250 amu; scan time, 1.227 sec.

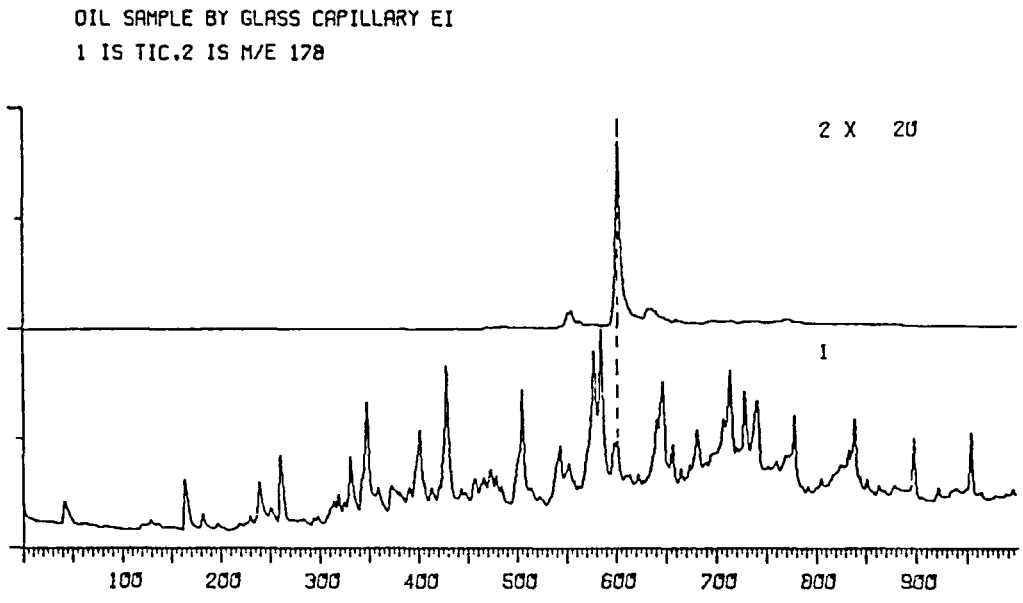


FIGURE 42. Total ion chromatogram overlapped with specific ion search for m/e, 178.

eluted from the column within the retention time window (±0.1 min) of the reference standard and exhibited the relative abundance ratio (±20%) observed in the pure compound. In most cases, this technique yielded positive identification. In certain instances co-eluting isomers with similar mass spectra could not be chromatographically resolved to allow unambiguous identification. Concentrations of individual PAHs which were detected ranged from 0.05 to about 8 ng/ℓ.

Strosher and Hodgson[111] analyzed Great Lakes water and sediments for their PAH content by GC/MS. The GC peaks which contained more than one PAH were resolved by repetitive oscillograph scanning over their respective GC elution times. For one peak, which consisted of contributions from three benzofluorene isomers, measurements were made of the relative abundances of fragment and doubly charged ions of each isomer. Rapid oscillograph scanning of the mass range from 70 to 320 m/e for the entire time span of the GC peak was sufficient to reveal the necessary differences in ionic abundance to determine the order of isomer elution. These studies, which could by means of elaborate computer methods[111] yield quantitative measurements of multicomponent peaks, were restricted to qualitative peak identifications.

Janini et al.[63] have investigated the use of BPhBT liquid crystal-packed columns in GC/MS analyses. Using the SIM mode at m/e of 252, it was possible to detect a sample injection of 4 ng of B(*a*)P. They stated that optimization of instrumental parameters could lower this limit to less than 1 ng. When the mass spectrometer was used in the normal mass scanning mode, 30 ng of B(*a*)P was the detection limit.

Detection limits for PAHs using column packings with lower levels of column bleed than BPhBT should be approximately 0.01 to 0.1 ng for normal mass scanning modes and about 1 to 10 pg for SIM modes.

The most important advantage of GC/MS over GC and HPLC for analyses of PAHs may be its cost-effectiveness. Finnigan et al.[112] and Budde and Eichelberger[113] have addressed this issue for the analyses of organics in the environment. Both groups concluded that GC/MS is less costly on a cost per analysis basis when a large number, approximately 100 or more, PAHs or other organics are to be determined. This result

was obtained by considering the capital costs of purchasing the necessary equipment to run the analyses as well as the operator costs and the overhead. Positive identification in GC cannot be made unless authentic samples are analyzed. The time and effort spent in this procedure results in a cost factor twice that of using a GC/MS system. In addition to presenting a detailed breakdown of comparison costs of GC vs. GC/MS, Finnigan et al.[112] found through an industrial survey that GC/MS gave more reliable determinations of PAHs than GC.

VI. SUMMARY

The type of sample which is to be analyzed and the number of compounds of analytical interest define the chromatographic approach to be used. A number of chromatographic methods have been developed for the analysis of the PAH fraction of the organic compounds isolated from a water sample. The number of PAHs can range from 6 to 200 compounds. When a cleanup procedure is used, the six WHO target PAHs can be successfully analyzed on a nanogram level by TLC, and it would be unnecessary to use HPLC, GC, or GC/MS to monitor just these six compounds. However, when the sample types grow in complexity and can include 20 or more PAHs, techniques such as HPLC, GC, or GC/MS must be considered.

HPLC has been the most successful means of separating isomeric PAHs. Unfortunately, at this time only about 20 PAH peaks can be resolved by HPLC without overlap. The Environmental Protection Agency has listed 16 PAHs on their list of Priority Pollutants,[27] and a complete resolution of these 16 compounds by HPLC has been achieved by Ogan et al.[27] Picogram amounts of PAH can be determined by HPLC coupled with fluorescence detection. Picogram amounts of PAHs can be determined. A certain amount of cleanup is necessary to isolate the PAHs from the other organics, but fluorescence detection provides additional selectivity.

The complete analysis of environmental samples with as many as 200 PAHs requires the use of capillary column GC. Although the establishment of a system of retention indices for capillary column GC by Lee et al.[75] is a step in the direction of total GC analysis without mass spectrometric identification, at the present time only computerized GC/MS systems are capable of analyzing complex environmental samples.

Any laboratory engaged in the analysis of environmental samples should have available TLC methods which can be used for both cleanup and analytical purposes. GC/MS analyses suffer some loss of sensitivity and selectivity towards PAHs as compared to HPLC, but a much broader range of sample complexity can be handled by this technique and this is our preferred method of analysis. A larger initial investment is necessary for GC/MS. Cost-effectiveness studies find GC/MS more economical than GC with conventional detectors.[112] A study of this type would probably also find HPLC less economical than GC/MS for samples with 200 or more PAHs. Development of HPLC/MS systems might eventually lead to a method rivaling GC/MS in broad-ranged applicability.

REFERENCES

1. Smith, S. R., Futoma, D. J., Smith, T. E., and Tanaka, J., *Crit. Rev. Anal. Chem.*, in press.
2. Touchstone, J. C. and Dobbins, M. F., *Practice of Thin-Layer Chromatography*, John Wiley & Sons, New York, 1978.
3. Nielsen, T., *J. Chromatogr.*, 170, 147 (1979).
4. Lankmayr, E. P. and Muller, K., *J. Chromatogr.*, 170, 139 (1979).
5. Daisey, J. M. and Leyko, M. A., *Anal. Chem.*, 51, 24 (1979).
6. Gearing, J. N., Gearing, P. J., Lytle, T. F., and Lytle, J. S., *Anal. Chem.*, 50, 1833 (1978).

7. Giger, W. and Schaffner, C., *Anal. Chem.*, 50, 243 (1978).
8. Giger, W. and Blumer, M., *Anal. Chem.*, 46, 1663 (1974).
9. Borneff, J., in *Advances in Environmental Science and Technology*, Vol. 8, Pitts, J. N., Metcalf, R. C., and Lloyd, A. C., Eds., John Wiley & Sons, New York, 1977.
10. Dunn, B. P., *Environ. Sci. Technol.*, 10, 1018 (1976).
11. Snyder, L. R., *J. Chromatogr.*, 12, 488 (1963).
12. Hurtubise, R. J., Phillip, J. D., and Skar, G. T., *Anal. Chim. Acta*, 101, 333 (1978).
13. Pierce, R. C. and Katz, M., *Anal. Chem.*, 47, 1743 (1975).
14. Katz, M., Sakuma, T., and Ho, A., *Environ. Sci. Technol.*, 12, 909 (1978).
15. Junk, G. A., Richard, J. J., Grieser, M. D., Witiak, D., Arguello, M. D., Vick, R., Svec, H. J., Fritz, J. S., and Calder, G. V., *J. Chromatogr.*, 99, 745 (1974).
16. Mainwaring, S. and McGuirk, J. M., Analytical and Technical Determination of Air Pollution, Symp., Melbourne, Australia, 1977, 84.
17. Bories, J., *J. Chromatogr.*, 130, 387 (1977).
18. Shiraishi, Y., Yamashita, T., and Shirotori, T., *Eisei Kagaku*, 23, 310 (1977).
19. Scholz, I. and Altmann, H. J., *Fresenius Z. Anal. Chem.*, 240, 81 (1968).
20. Basu, D. K. and Saxena, J., *Environ. Sci. Technol.*, 12, 795 (1978).
20a. Woidich, H., Pfannhauser, W., Blaicher, G., and Tiefenbacher, K., *Chromatographia*, 10, 140 (1977).
21. Kaschari, D. T. and Reiter, R., *Fresenius Z. Anal. Chem.*, 292, 141 (1978).
22. Pre-Symposium Notes, 4th Int. Symp. Polycyclic Aromatic Hydrocarbons, Columbus, Ohio, October 2 to 4, 1979.
23. Snyder, L. R., Dolan, J. W., and Gant, J. R., *J. Chromatogr.*, 165, 3 (1979).
24. Dolan, J. W., Gant, J. R., and Snyder, L. R., *J. Chromatogr.*, 165, 31 (1979).
25. Grushka, E. and Kitka, E. J., *Anal. Chem.*, 49, 1004 A (1977).
26. Wise, S. A., Chesler, S. N., Hertz, H. S., May, W. E., Bonnett, W. J., Brown, J. M., Guenther, F. R., Hilpert, L. R., and Parris, R. M., Normal and reverse phase liquid chromatographic separations of polycyclic aromatic hydrocarbons paper presented at the 4th Int. Symp. on Polycyclic Aromatic Hydrocarbons, Columbus, Ohio, October 2 to 4, 1979.
27. Ogan, K., Katz, E., and Slavin, W., *Anal. Chem.*, 51, 1315 (1979).
28. Ogan, K., Katz, E., and Slavin, W., *Chromatogr. Newsl.*, 7, 9 (1979).
29. Ogan, K., Katz, E., and Slavin, W., *J. Chromatogr. Sci.*, 16, 517 (1978).
30. Das, B. S. and Thomas, G. H., *Anal. Chem.*, 50, 967 (1978).
31. Sorrell, R. K., Dressman, R. C., and McFarren, E. F., paper presented at the Water Quality Technology Conf., Kansa City, Mo., December 5 to 6, 1977.
32. Fox, M. A. and Staley, S. W., *Anal. Chem.*, 48, 992 (1976).
33. Dong, M., Locke, D. C., and Ferrand, E., *Anal. Chem.*, 48, 368 (1976).
34. Krstulovic, A. M., Rosie, D. M., and Brown, P. R., *Anal. Chem.*, 48, 1383 (1976).
35. Krstulovic, A. M., Rosie, D. M., and Brown, P. R., *Am. Lab.*, 9, 11 (1977).
36. Euston, C. B. and Baker, D. R., *Am. Lab.*, 11, 91 (1979).
37. Kasiske, D., Klinkmuller, K. D., and Sonneborn, M., *J. Chromatogr.*, 149, 703 (1978).
38. Chmielowiec, J. and Sawatzky, H., *J. Chromatogr. Sci.*, 17, 245 (1979).
39. Hunt, D. C., Wild, P. J., and Crosby, N. T., *J. Chromatogr.*, 130, 320 (1977).
40. Hunt, D. C., Wild, P. J., and Crosby, N. T., *Water Res.*, 12, 643 (1978).
41. Crathorne, B. and Fielding, M., *Proc. Anal. Div. Chem. Soc.*, 15, 155 (1978).
42. Lochmuller, C. H. and Amoss, C. W., *J. Chromatogr.*, 108, 85 (1975).
43. Vespalec, R., *J. Chromatogr.*, 118, 47 (1976).
44. Popl, M., Dolansky, V., and Mostecky, J., *J. Chromatogr.*, 117, 117 (1976).
45. Popl, M., Dolansky, V., and Mostecky, J., *J. Chromatogr.*, 91, 649 (1974).
46. Popl, M., Dolansky, V., and Coupek, J., *J. Chromatogr.*, 130, 195 (1977).
47. Wise, S. A., Chesler, S. N., Hertz, H. S., Hilpert, L. R., and May, W. E., *Anal. Chem.*, 49, 2306 (1977).
48. Slavin, W., William, A. T. R., and Adams, R. F., *J. Chromatogr.*, 134, 121 (1977).
49. Christensen, R. G. and May, W. E., *J. Liq. Chromatogr.*, 1, 385 (1978).
50. Eisenbeiss, F., Hein, H., Joester, R., and Naundorf, G., *Chromatogr. Newsl.*, 6, 8 (1978).
51. Arpino, P. J. and Guiochon, G., *Anal. Chem.*, 51, 682A (1979).
52. van Deemter, J. J., Zuiderweg, F. J., and Klinkenberg, A., *Chem. Eng. Sci.*, 5, 271 (1956).
53. Gas-Thin Layer-Liquid Chromatography — Chemicals and Accessories, Catalog 18, Analabs, Inc., North Haven, Conn. 1976.
54. Wells, D. E., *Dyn. Mass. Spectrom.*, 5, 89 (1978).
55. Thomas, R. S., Lao, R. C., Wang, D. T., Robinson, D., and Sakuma, T., Polynuclear aromatic hydrocarbons, in *Carcinogenesis*, Vol. 3, Jones, P. W. and Freudenthal, R. I., Eds., Raven Press, New York, 1978.

56. **Lao, R. C., Thomas, R. S., and Monkman, J. L.,** Polynuclear aromatic hydrocarbons, in *Carcinogenesis,* Vol. 1. Jones, P. W. and Fruedenthal, R. I., Eds., Raven Press, New York, 1976, 271.
57. **Lao, R. C., Thomas, R. S., Oja, H., and Dubois, L.,** *Anal. Chem.,* 45, 908 (1973).
58. **Lao, R. C., Thomas, R. S., and Monkman, J. L.,** *J. Chromatogr.,* 112, 681 (1975).
59. **Strosher, M. T. and Hodgson, G. W.,** *ASTM Special Tech. Publ.,* 573, 259 (1975).
60. **Strand, J. W. and Andren, A. W.,** *Anal. Chem.,* 50, 1508 (1978).
61. **Janini, G. M., Johnston, K., and Zielinski, W. L., Jr.,** *Anal. Chem.,* 47, 670 (1975).
62. **Janini, G. M., Muschik, G. M., and Zielinski, W. L., Jr.,** *Anal. Chem.,* 48, 809 (1976).
63. **Janini, G. M., Muschik, G. M., Schroer, J. A., and Zielinski, W. L., Jr.,** *Anal. Chem.,* 48, 1879 (1976).
64. **Janini, G. M., Shailch, B., and Zielinsky, W. L., Jr.,** *J. Chromatogr.,* 132, 136 (1977).
65. **Novotny, M.,** *Anal. Chem.,* 50, 16A (1978).
66. **Grob, K. and Grob, G., in** *Identification and Analysis of Organic Pollutants in Water,* Keith, L. H., Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1975, 75.
67. **Cram, S. P. and Yang, F. S.,** Symposium on Analytical Chemistry of Petroleum Hydrocarbons in Marine/Aquatic Environment, American Chemical Society Meeting, September 1978, 845.
68. **Lee, M. L., Vassilaros, D. L., Phillips, L. V., Hercules, D. M., Asumaya, H., Jorgenson, J. W., Maskarinec, M. P., and Novotny, M.,** *Anal. Lett.,* 12 (A2), 191 (1979).
69. **Meuser, J. M., Bjorseth, A., Moore, F. R., Strup, P. E., and Wilkinson, J. E.,** Abstract from 4th Int. Symp. Polynuclear Aromatic Hydrocarbons, Columbus, Ohio, October 2 to 4, 1979.
70. **Schomburg, G., Dielmann, R., Borwitzky, H., and Husmann, H.,** *J. Chromatogr.,* 167, 337 (1978).
71. **Borwitzky, H. and Schomburg, G.,** *J. Chromatogr.,* 170, 99 (1979).
72. **Schomburg, G., Husmann, H., and Borwitzky, H.,** *Chromatographia,* 12, 651 (1979).
73. **Blomburg, L. and Wannman, T.,** *J. Chromatogr.,* 148, 379 (1978).
74. **Dandeneau, R., Bente, P., Rooney, T., and Hiskes, R.,** *Am. Lab.,* 11, 61 (1979).
75. **Lee, M. L., Vassilaros, D. L., White, C. M., and Novotny, M.,** *Anal. Chem.,* 51, 768 (1979).
76. **Bjørseth, A.** *Anal. Chim. Acta,* 94, 21 (1977).
77. **Bjørseth, A.,** Polynuclear aromatic hydrocarbons, in *Carcinogenesis,* Vol. 3, Jones, P. W. and Freudenthal, R. I., Eds., Raven Press, New York, 1978, 75.
78. **Bjørseth, A. and Eklund, G.,** *Anal. Chim. Acta,* 105, 119 (1979).
79. **Bjørseth, A.,** *Polynuclear Aromatic Hydrocarbons,* Jones, P. W. and Leber, P., Eds., Ann Arbor Science Publishers, Ann Arbor, Mich., 1979, 371.
80. **Lao, R. C. and Thomas, R. S.,** *Polynuclear Aromatic Hydrocarbons,* Jones P. W. and Leber, P., Eds., Ann Arbor Science Publishers, Ann Arbor, Mich., 1979, 429.
81. **Blomberg, L., Buijlen, J., Gaudzik, J., and Wannmann, T.,** *Chromatographia,* 11, 521 (1978).
82. **Stenberg, U., Alsoberg, T., Blomberg, L., and Wannmann, T.,** Polynuclear Aromatic Hydrocarbons, Jones P. W. and Leber, P., Eds., Ann Arbor Science Publishers, Ann Arbor, Mich., 1979, 313.
83. **Blomberg, L. and Wannmann, T.,** *J. Chromatogr.,* 168, 81 (1979).

83a. Manufacturers chromatogram, J & W Scientific, Orangevale, Calif., 1980.

84. **Maskarinec, M. P. and Olerich, G.,** *Anal. Chem.,* 52, 588 (1980).
85. **Grob, R. L., Ed.,** *Modern Practice of Gas Chromatography,* John Wiley & Sons, New York, 1977.
86. **Giger, W. and Schaffner, C.,** *Anal. Chem.,* 50, 243 (1978).
87. **Acheson, M. A., Harrison, R. M., Perry, R., and Wellings, R. A.,** *Water Res.,* 10, 207 (1976).
88. **Burchill, P., Herod, A. A., and James, R. G.,** Polynuclear aromatic hydrocarbons, in *Carcinogenesis,* Vol. 3, Jones, P. W. and Freudenthal, R. I., Eds., Raven Press, New York, 1978, 35.
89. **Radecki, A., Lamparczyk, H., Grzybowski, J., and Kalkiewicz, J.,** *J. Chromatogr.,* 150, 527 (1978).
90. **Freed, D. J. and Faulkner, L. R.,** *Anal. Chem.,* 44, 1194 (1972).
91. **Robinson, J. W. and Goodbread, J. P.,** *Anal. Chim. Acta,* 66, 239 (1973).
92. **Caddy, D. E. and Meek, D. M.,** *Proc. Anal. Div. Chem. Soc.,* 13, 45 (1976).
93. **Cooney, R. P. and Winefordner, J. D.,** *Anal. Chem.,* 49, 1057 (1977).
94. **Driscoll, J. N. and Spaziani, F. F.,** *Res. Dev.,* 27, 50 (1976).
95. **Driscoll, J. N.,** *Am. Lab.,* 8, 71 (1976).
96. **Driscoll, J. N.,** *J. Chromatogr.,* 134, 49 (1977).
97. **Driscoll, J. N., Ford, J., Jaramillo, L., Becker, J. H., Hewitt, G., Marshall, J. K., and Onishuk, F.,** *Am. Lab.,* 10, 147 (1978).
98. **Oyler, A. R., Bodenner, D. L., Welch, K. J., Liukkonen, R. J., Carlson, R. M., Kopperman, H. L., and Caple, R.,** *Anal. Chem.,* 50, 837 (1978).
99. HNU Systems Inc., Cambridge, Mass., Form PID/210778.
100. **Driscoll, J. N., Marshall, J. K., Jaramillo, L. F., Hewitt, G., Alongi, V.,** *Am. Lab.,* 12, 84 (1980).
101. **Driscoll, J. N., Ford, J., Jaramillo, L. F., and Gruber, E. T.,** *J. Chromatogr.,* 158, 171 (1978).
102. **Hester, N. H. and Meyer, R. A.,** *Environ. Sci. Technol.,* 13, 107 (1979).

103. **McFadden, W. H.,** *J. Chromatogr. Sci.,* 17, 2 (1979).
104. **DeBrauw, M. C. T.,** *J. Chromatogr.,* 165, 207 (1979).
105. **Fenselau, C.,** *Anal. Chem.,* 49, 563A (1977).
106. **Clerc, J. T., Kutter, M., Reinhard, M., and Schwarzenbach, R.,** *J. Chromatogr.,* 123, 271 (1976).
107. **Naegeli, P. R. and Clerc, J. T.,** *Anal. Chem.,* 46, 739A (1974).
108. **McGuire, J. M., Carter, M. H., and Alford, A. L.,** in *Identification and Analysis of Organic Pollutants in Water,* Keith, L. H., Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1975, 175.
109. **Finnigan, R. E. and Knight, J. B.,** in *Identification and Analysis of Organic Pollutants in Water,* Keith, L. H., Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1975, 185.
110. **Benoit, F. M., Lebel, G. L., and Williams, D. T.,** *Int. J. Environ. Anal. Chem.,* 6, 277 (1979).
111. **Strosher, M. T. and Hodgson, G. W.,** *ASTM Special Tech. Publ.,* 573, 259 (1975).
112. **Finnigan, R. E., Hoyt, D. W., and Smith, D. E.,** *Environ. Sci. Technol.,* 13, 534 (1979).
113. **Budde, W. L. and Eichelberger, J. W.,** *Anal. Chem.,* 51, 567A (1979).