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Analysis of Aromatic Compounds in Water Using Fluorescence and Phosphorescence

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THE ANALYSIS OF AROMATIC COMPOUNDS IN WATER USING FLUORESCENCE AND PHOSPHORESCENCE

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INTRODUCTION

The presence of small amounts of polynuclear aromatic hydrocarbons (PNA) in many areas of the environment has focused attention on the development of improved analytical measurements for their determination. Much of this interest can be attributed to the fact that a substantial number of PNA have been shown to be highly carcinogenic.

The major source of PNA in the environment is the incomplete combustion of coal and other fuels. As such PNA compose a significant percentage of chimney soot and coaltar pitch. Trace amounts of PNA have been found in association with a wide variety of other substances throughout the environment. Although most of these sources, such as airborne particulate matter or cigarette smoke, can be traced directly or indirectly to incomplete combustion, there is sufficient evidence to demonstrate that trace amounts of PNA are synthesized by naturally occurring organisms.

The presence of PNA in air particulates has been firmly established and it is reasonable to assume that some are transported into the natural water system. The purpose of this work was to develop a method for the determination of trace amounts of PNA present in natural water. The analytical scheme as envisioned initially was composed of four major steps. (1) Continuous liquid-liquid extraction of 5 liters of water with n-pentane followed by concentration of the extract to several milliliters. (2) Column chromatography of the resultant concentrate on acidic alumina to remove interfering basic components. (3) Thin-

layer chromatography (TLC) of the concentrated eluent to separate the individual PNA. (4) Quantitative fluorescence analysis of the separated compounds. Modifications of this preliminary procedure are discussed below.

DEVELOPMENT OF THE METHOD

Four commonly occurring PNA were chosen as reference compounds for the present work. They were benzo(a)pyrene (BaP), dibenz(a,h) anthracene (DiBahA), benzo(b)fluorene (BbF), and fluoranthene (Fluor).

All solvents used were of the highest quality commercially available. New batches of solvent were checked for purity fluorometrically. Distillation was used for further purification when necessary. The highest quality chemicals were purchased from commercial supply houses and their purity checked using TLC and fluorescence.

LIQUID-LIQUID EXTRACTION

Concentration of the trace amounts of PNA is necessary before analysis. Liquid-liquid extraction was chosen as the preliminary step in the present method. Continuous extraction with pentane was used for the majority of this work and proved to be quite satisfactory in spite of being time consuming. Batch extractions were investigated later and found to give excellent results if certain precautions were taken. Pesticide Analysis Grade pentane (Fisher Scientific Company) was used exclusively as the extracting solvent.

Continuous Extraction

Three continuous extractors (A,B, and C), each of slightly different design, were used. Each extractor was capable of holding approximately 4500 ml of water. Flat bottoms were provided in extractors B and C to accommodate a 5 cm Teflon-coated magnetic stirring bar. 200 ml of pentane was placed in a round-bottom flask attached to the side-arm of the extractor.

The course of each extraction was followed fluorometrically. Aliquots of pentane were removed periodically from the side-arm flask and the fluorescence measured. If necessary, pure pentane was added to the flask prior to removal of the aliquot so that a constant volume was being sampled. The extraction was assumed to be complete when the fluorescence remained essentially constant for 4 hours.

A summary of times required for complete extraction is presented in Table I. These figures are slightly inflated because they were compiled from total extraction times which included the 4 hours allowed for the fluorescence to become constant. In some cases as much as 12 hours had elapsed between the final two aliquots. The data in Table I clearly shows the effect of stirring on extraction time. The mean times for extractors B and C, in which stirring was employed, are significantly lower than that for extractor A which was not stirred. By increasing the rate of stirring, the last 16 extractions with B and C were each completed in approximately 24 hours.

Extraction efficiency for the four reference PNA were determined with extractors B and C. Synthetic samples of PNA dissolved in distilled water were used. Because of their extremely low solubility in water, PNA were introduced directly by means of a dilute ethanol solution into the extraction water. The percentage of ethanol in the water was always kept below 0.1%. Percent recoveries were calculated by comparison of the fluorescence of the extract with that of a similar solution of known concentration.

TABLE I

Summary of Time Required for Complete

Extractions of PNA with Extractors A, B, and C

Extractor	No. of Extractions	Range (hours)	Mean (hours)
A	22	29-101	54
B	20	16-75	42
C	15	16-68	37

The percent recovery data for the form reference PNA are shown in Table II. The percent recovery values are the means from triplicate determinations. All of the compounds were recovered with greater than 90% efficiency. These values are quite good considering the possibility of losses due to adsorption on glass surfaces. The major drawback to continuous extraction is the length of time required.

Batch Extraction

The feasibility of using batch extractions was studied in an effort to greatly reduce extraction time. These extractions were carried out in 4 liter separatory funnels. Three liters of water were extracted with 100 ml of pentane by shaking for 5 minutes. Recovery studies were conducted in the same manner as with the continuous extractions. Preliminary studies indicated greater than 90% recovery for each compound. However, when the procedure was applied to a real natural water sample, a very stable emulsion formed which prohibited quantitative separation of the two phases.

Efforts to break up these emulsions by the addition of salt and also the suspending agents, Superfloc and Aerofloc (American Cyanamide Company) were unsuccessful. It was necessary, therefore, to prevent the formation of the emulsion. This was possible by gently swirling the funnel instead of shaking it vigorously. Preliminary tests indicated that a longer extraction time was necessary.

A series of batch extractions were performed in which 3 liters of a known PNA water solutions were gently swirled with 100 ml of pentane for 10 minutes. The results are shown in Table III. The concentration of each PNA was less than 400 parts per trillion in each case.

Table II.

Continuous Extraction Efficiency for Reference PNA

	BaP		BbF		DiBahA		Fluor	
Extractor	B	C	B	C	B	C	B	C
Concentration (ppt)	412	424	375	386	518	533	486	499
Mean % recovery	97.3	97.3	95.0	94.1	94.4	90.6	99.7	96.9
Relative standard deviation	3.1	3.8	3.2	3.4	1.1	1.1	3.6	3.5

Table III.

Batch Extraction Efficiency for Reference PNA

	Mean % Recovery	Relative Standard Deviation
BaP	99.3	1.76
BbF	98.8	2.36
DiBahA	97.0	1.83
Fluor	97.5	2.08

The percent recovery values are the means from 4 replicate determinations. The recovery values are slightly higher and the relative standard deviations slightly lower than those obtained using continuous extraction and constitute an improvement both in amount recovered and time required.

It would thus seem that batch extraction would be preferred to continuous extraction in most cases. Continuous extraction, on the other hand, might still be necessary if emulsion formation cannot be prevented with some samples.

PRELIMINARY SEPARATION

Before TLC separation of the individual PNA and subsequent fluorescence analyses, it is desirable to remove as many interferring fluorescent substances as possible. This is particularly true of the basic heterocycles which not only are fluorescent but also have migration distances comparable to those of most PNA. Several separation systems were investigated to find one which would remove most interferring basic compounds with a minimum loss of PNA.

During the preliminary stages of this work, column chromatography on acidic alumina was used for preliminary separation. Although recoveries of PNA were excellent, the approach was abandoned because of the time involved and the necessity of concentrating large volumes of solvent. A variety of other procedures, including extraction with dilute HCl, extraction with 72% perchloric acid, and batch contact with both activated and deactivated acidic alumina, were investigated using benzo(a)pyrene and carbazole as representative PNA and basic heterocyclic compounds,respectively. All of these approaches were abandoned because of their inability to remove basic components while leaving most of the PNA in the pentane layer.

The procedure yielding the best results consisted of batch extracting 75 ml of a PNA pentane solution one time with 25 ml of 72% H_2SO_4. The results of a study using the system are shown in Table IV. The efficiency of removing carbazole from the pentane solution is greater than 90% from all solutions except the lowest concentration of 5 ppb. Even at this low concentration, approximately 70% of the carba-

TABLE IV

Summary of Recovery Data for Preliminary Separation with 72% Sulfuric Acid

Benzo(a)pyrene			Carbazole		
Concentration	% Recovery	Mean	Concentration	% Recovery	Mean
1 ppb	95.7, 91.0	93.4	5 ppb	31.5, 30.9	31.2
10	101 , 97.3	99.2	50	4.8, 8.4	6.6
100	102 , 101	101.5	500	4.1, 12.4	8.3
1000	96.5, 100	98.3	5000	3.0, 3.0	3.0

Conditions: 75 ml of benzo(a)pyrene-carbazole (1:5) solution extracted one time with 25 ml of 72% sulfuric acid. % recovery data represent the % of each compound remaining in the pentane layer.

zole was removed. The 30% carbazole remaining in the pentane layer is tolerable in the overall method because at this low concentration, the amount of carbazole spotted on the TLC plate would not constitute a serious interference. The sulfuric acid extraction was used in all subsequent analyses of unknown samples.

THIN-LAYER CHROMATOGRAPHY

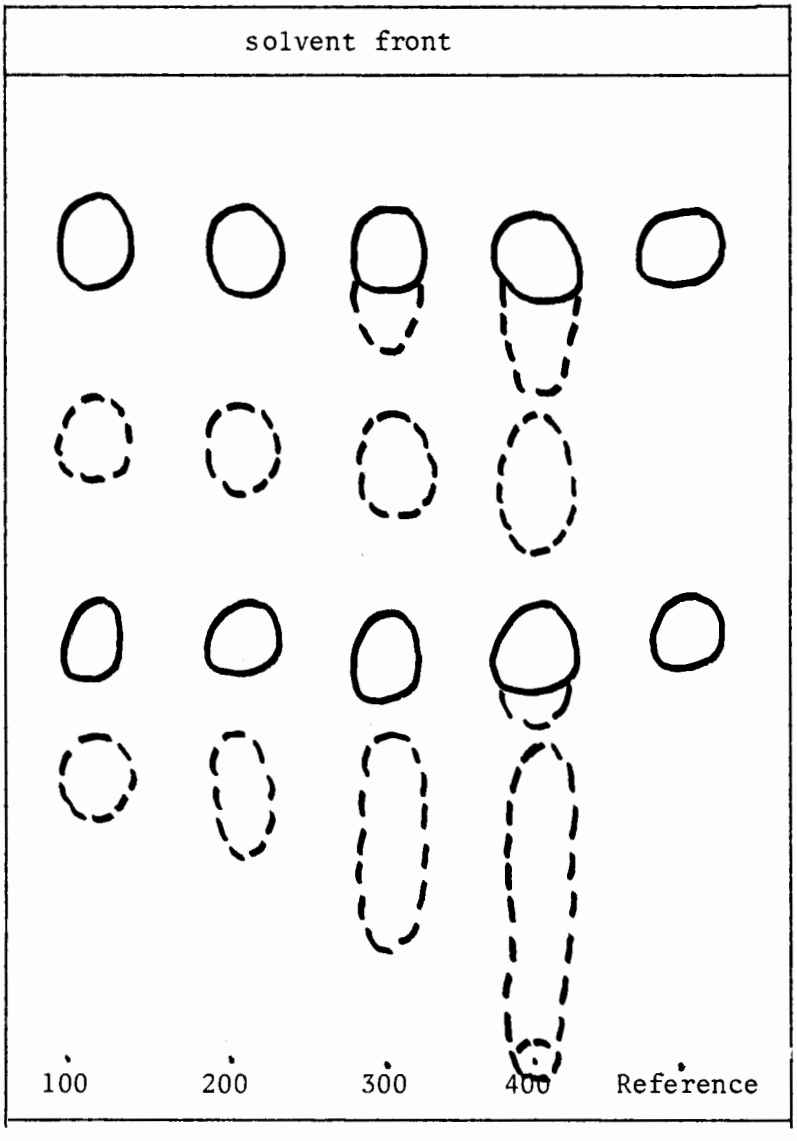
After separation of basic interferences, it is necessary to further separate the individual PNA for fluorescence analysis, TLC is an ideal method for PNA separation. Many TLC systems have appeared in the literature for separating PNA. Excellent separations have been achieved for most PNA. The TLC systems investigated in this study are summarized in Table V. The best separations were achieved using a cellulose adsorbent developed with 50% aqueous dimethylformamide (DMF). A typical chromatogram of the 4 reference PNA is shown in Figure 1. This system was used exclusively in the remainder of the work. All cellulose-DMF plates were developed using the sandwich technique. The precoated cellulose Baker-flex sheets were taped to a glass plate for insertion into the chamber. Samples were applied with Lang-Levy micropipets as spots 1.5 cm from the bottom of the plate and approximately 1.9 cm from each other. Ethanol was used as the solvent in which the PNA were dissolved because of the high volatility of pentane which made quantitative application impossible. Because of the extreme light sensitivity of most PNA, the entire apparatus was covered with a black cloth during development. Development was continued until the solvent front had travelled 15 cm. The average development time was approximately 4 hours.

TABLE V

Summary of TLC Systems Investigated

Adsorbent	Eluent	
Silica Gel GF_{254}	Pentane-benzene	(1:1)
	Hexane-benzene	(1:1)
	Hexane	
	Carbon tetrachloride	
Silica Gel PF_{254}	Pentane-benzene	(1:1)
	Hexane-benzene	(1:1)
	Hexane	
	Carbon tetrachloride	
Alumina GF_{254}	Pentane-ether	(19:1)
	Hexane	
	Carbon tetrachloride	
Cellulose MN300	Dimethylformamide-water	(3:1)
	Dimethylformamide-water	(1:3)
	Dimethylformamide-water	(3:2)
	Dimethylformamide-water	(1:1)

Figure 1. Typical separation of reference compounds on cellulose as seen under 254 nm light.



Developer: 50% aqueous dimethylformamide
Time: 3 hours, 50 minutes
Reference: 50 ng each of BaP and Fluoranthene
_____: Intense fluorescence
- - - - -: Weak fluorescence

Location of visible spots on developed chromatograms was accomplished under UV illumination. Both a short wave and a long wave lamp were used giving peak intensities at 254 and 365 nm, respectively. The separated reference compounds were more distinct under 254 nm than 365 nm excitation light on cellulose layers. Illumination of the plates with UV light was kept as short as possible to minimize spot decomposition. The minimum amount of material necessary for visual observation varied among the four reference compounds. The observed fluorescence is dependent upon both the natural fluorescence quantum efficiency and each compound's adsorptivity at 254 nm. Minimum observable amounts of benzo(a)pyrene and fluoranthene were on the order of 10 ng while those for benzo(b)fluorene and dibenz(a,h)anthracene were about 30 ng.

Before the TLC scanning attachment was purchased, the removal of PNA from cellulose plates, prior to fluorometric quantitation, was investigated. These methods involved scraping the adsorbent layer containing the spot as well as the use of a vacuum spot collector followed by ethyl ether extraction of the adsorbent layer. Recoveries were very poor and the increased fluorescence background caused by concentration of the ether extract prohibited the quantitation of small amounts of PNA. This method was abandoned with the acquisition of the Farrand TLC scanner.

FLUORESCENCE ANALYSIS

Preliminary fluorescence work was done using a spectrofluorometer designed and built in the laboratory. This instrument was dismantled with the acquisition of the Farrand MK-1 Spectrofluorometer which was used for the majority of the work.

The Farrand MK-1 Spectrofluorometer employed a 150 watt zenon arc lamp for fluorescence excitation. This lamp provided a continuous output throughout the ultraviolet and visible regions of the spectrum. Both the excitation and emission monochromators were of the modified Czerny-Turner type having a wavelength range from 200 to 700 nm. Wavelength accuracy was $\pm$ 2 nm. In all solution work slit widths were chosen to produce a band pass of 5 nm.

Radiation emerging from the excitation monochromator was focused on a 1 cm square fused quartz cell and the fluorescence was detected at a right angle by a 1P28 photomultiplier tube after passing through the emission monochromator. After amplification, the output was recorded on a Houston Onmigraphic Corporation Model HR-96T x-y recorder.

A Turner Filter Fluorometer Model 110 was also used. It is a null-point instrument incorporating a general purpose ultraviolet lamp with appropriate filters. All measurements were made using a number 7-60 excitation filter which peaks at 360 nm and a number 2A emission filter which passes wavelengths longer than 415 nm. Samples were contained in a cylindrical 1 cm quartz cell.

All solution fluorescence measurements were made in Pesticide Analysis Grade n-pentane, the same solvent which was used in the extraction experiments. Each new batch of solvent was checked fluorometrically to assure the absence of contamination.

For quantitative fluorescence work, two different reference systems were employed. Comparison with a standard reference material is necessary to correct for variations in the light source or photomultiplier. Initially, the fluorescence emission at 450 nm from a 1 ppm solution of quinine sulfate in 0.1 $\underline{N}$ H_2SO_4 was used. The solution was excited with 350 nm light. Subsequent work was performed using a 1 cm solid pyrex reference cell obtained from Farrand Optical Company. The excitation monochromator was set at 350 nm, and the corresponding scatter peak at 350 nm was recorded. Fluorescence excitation and emission spectra for the five reference compounds used in this study are shown in Figures 2 - 6.

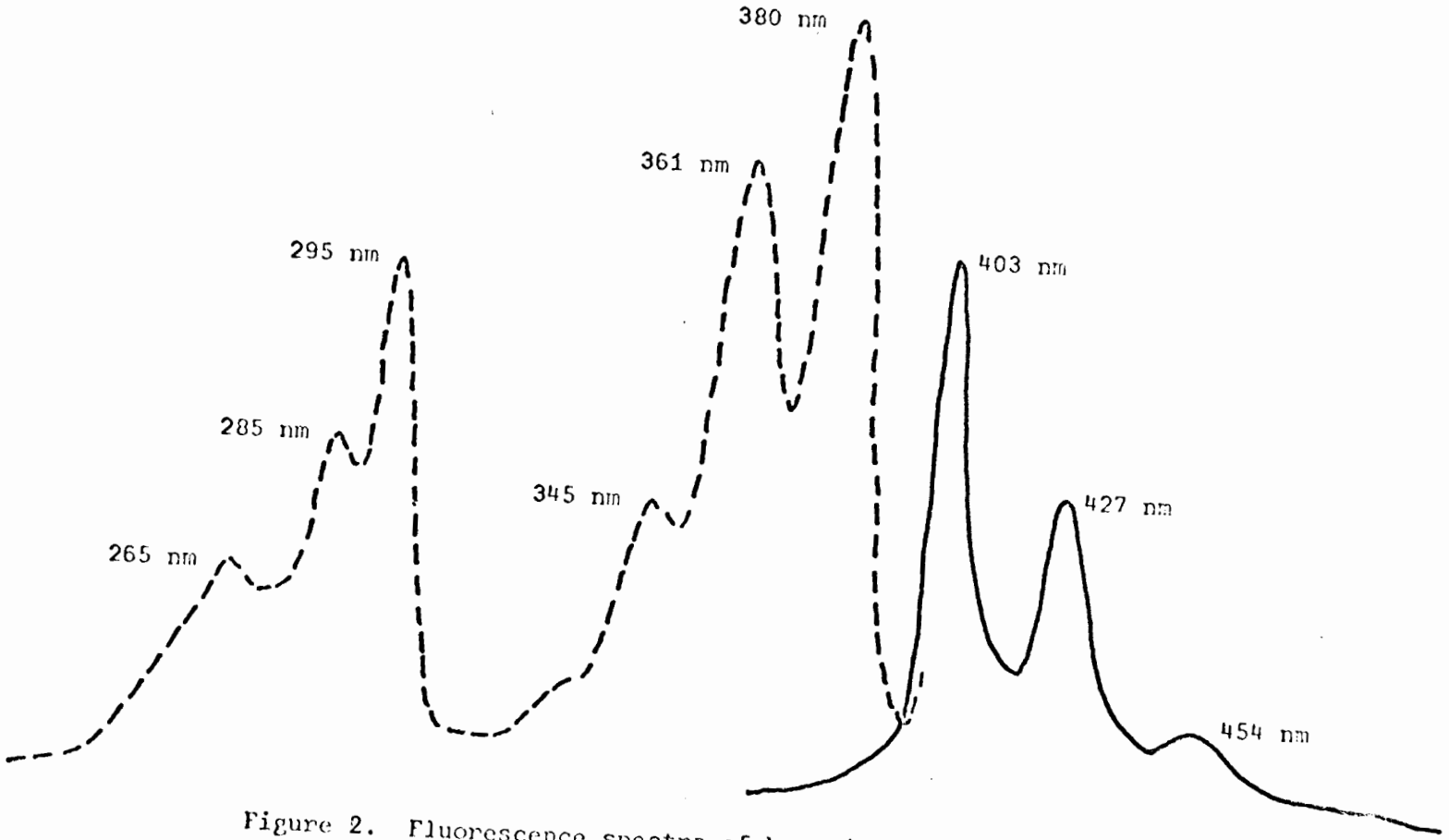


Figure 2. Fluorescence spectra of benzo(a)pyrene in pentane

- - - - Excitation spectrum at emission wavelength 403 nm

———— Emission spectrum at excitation wavelength 295 nm

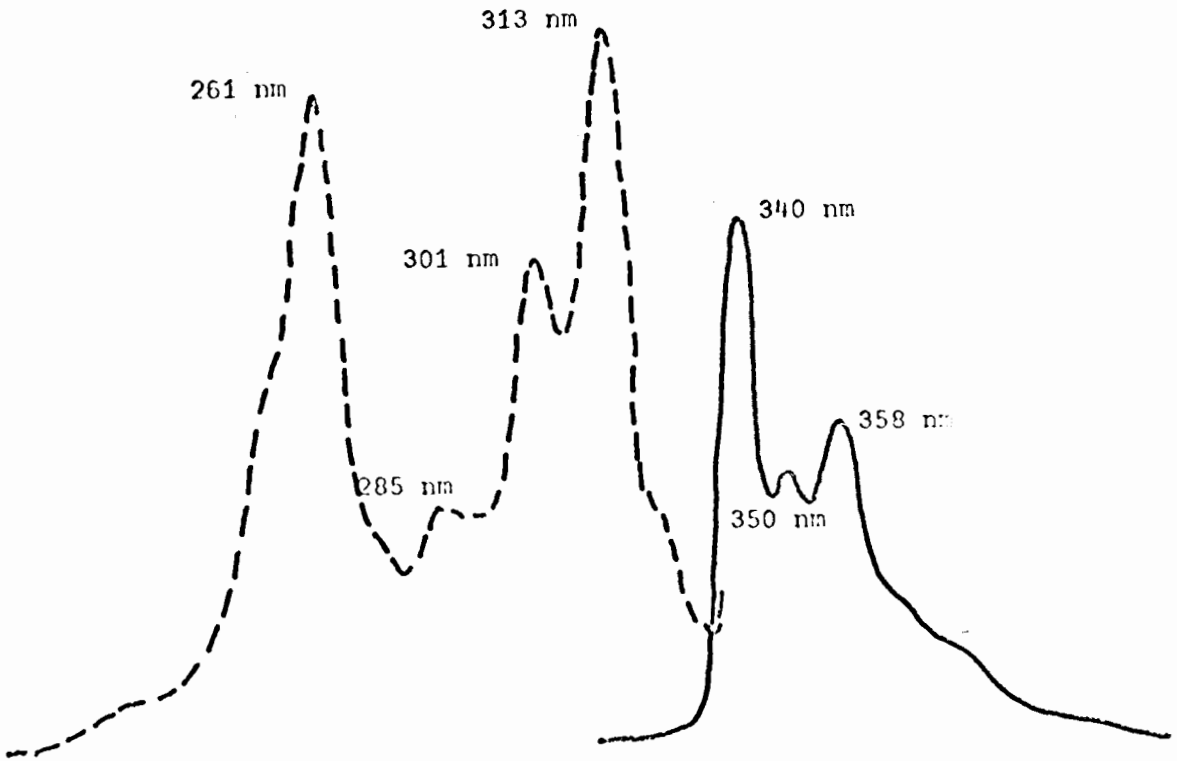


Figure 3. Fluorescence spectra of benzo(b)fluorene in pentane

- - - - Excitation spectrum at emission wavelength 340 nm

——— Emission spectrum at excitation wavelength 301 nm

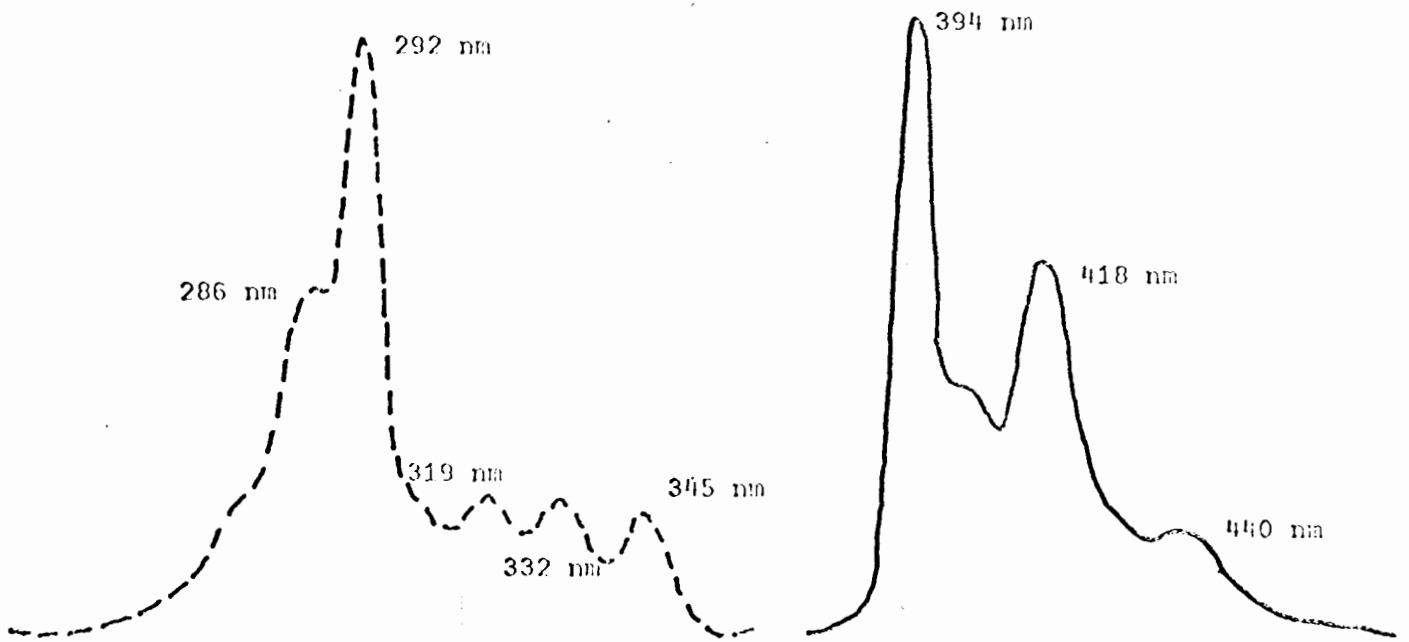


Figure 4. Fluorescence spectra of dibenz(a,h)anthracene in pentane.

- - - - Excitation spectrum at emission wavelength 394 nm

——— Emission spectrum at excitation wavelength 292 nm

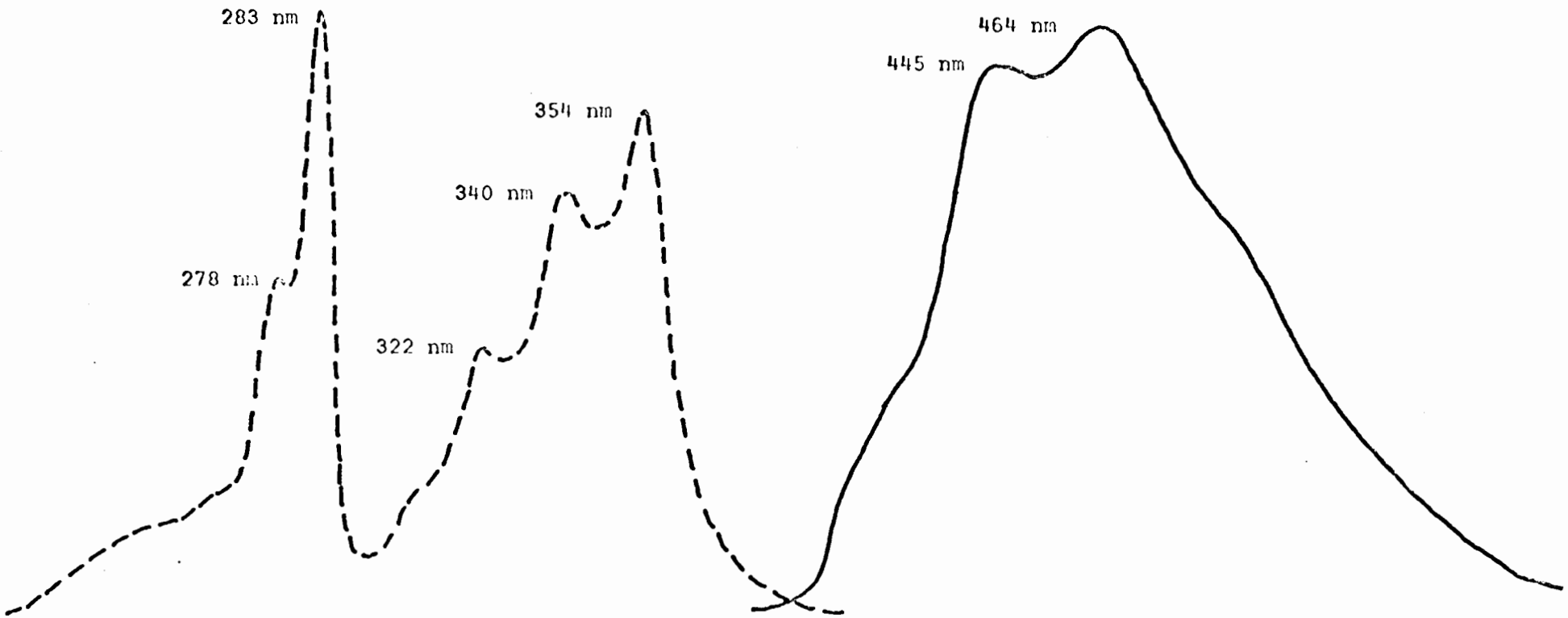


Figure 5. Fluorescence spectra of fluoranthene in pentane

- - - - Excitation spectrum at emission wavelength 464 nm

——— Emission spectrum at excitation wavelength 283 nm

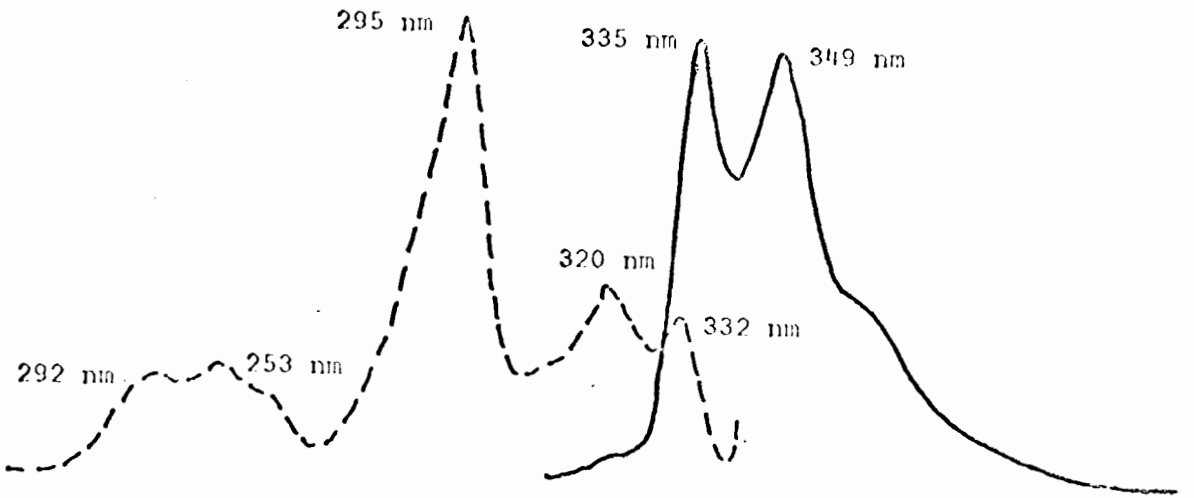


Figure 6. Fluorescence spectra of carbazole in pentane

– – – – Excitation spectrum at emission wavelength 349 nm

——— Emission spectrum at excitation wavelength 295 nm

FLUOROMETRIC TLC SCANNING

A TLC scanner was purchased from Farrand Optical Company and affixed atop the Farrand MK-1 Spectrofluorometer. The scanner is a 50 x 48 x 11 cm light-tight metal box containing a movable carriage which holds any size TLC plate up to 20 x 20 cm. The plate is held in place in the carriage, adsorbent layer facing downward, with magnets placed around its edge. The carriage is moved by a reversible, variable speed synchronous motor in one direction and by a manually controlled calibrated dial perpendicular to the first direction. Scanning rates may be selected from 10 pre-set values ranging from 0.4 to 12 inches per minute. Transmission of the light beam from the excitation monochromator to the TLC plate and the reflected fluorescence emission from the plate to the emission monochromator is accomplished by a series of lenses and mirrors built into a small adaptor which fits into the sample compartment of the spectrofluorometer. This unit is easily installed and makes it possible to change from TLC scanning to solution fluorescence and vice versa in a few minutes. The image of the light beam on the TLC plate is defined by interchangeable adaptor slits of various sizes which fit on the adaptor. A slit with dimensions 1.5 x 13 mm was found to provide sufficient sensitivity with good resolution and was used throughout this work.

Identification of PNA in unknown extracts was based primarily on R_B values (the migration distance of the compound relative to that of benzo(a)pyrene) and fluorescence spectra obtained directly from the TLC adsorbent. R_B values are characteristic of a particular compound

for a given TLC system. R_B values were calculated from the migration distances of individual spots measured by scanning the developed chromatogram parallel to the direction of development. The output from the photomultiplier tube was applied to a strip chart recorder to obtain a permanent record of each scan. The speed of both the scanning carriage and the recorder were synchronized at the rate of one inch per minute so that migration distances could be read directly from the chart. In most separations, sufficient fluorescent material remained at the origin to be recorded as a peak from which migration distances could be measured. In those cases where the origin was nonfluorescent it was necessary to visually position the adaptor slit directly over the origin and begin the scan at a predetermined position on the strip chart. The mean R_B values of the form reference PNA as determined on 15 chromatograms from the present work are shown in Table VI.

The spectrofluorometer scanning system also permitted the measurement of fluorescence spectra directly from the TLC adsorbent. After location of the desired spot, the position of the scanner was adjusted to obtain the maximum fluorescence signal. Both excitation and emission spectra were then obtained by scanning with the appropriate monochromator.

For the four reference compounds studied, all fluorescence spectra measured directly from the cellulose layer showed no wavelength shifts from those measured in pentane solution. This is reasonable considering the non-polar nature of the cellulose adsorbent.

Table VI

R_B Values of Reference Compounds

	R_B	Relative Standard Deviation
Dibenz(a,h)anthracene	0.66	8.4%
Benzo(a)pyrene	1.00	5.1%
Benzo(b)fluorene	1.40	4.1%
Fluoranthene	1.90	2.9%

The smallest quantity necessary to produce a resolvable fluorescence emission spectrum was determined for the reference compounds as shown below:

Dibenz(a,h)anthracene	5 ng
Benzo(a)pyrene	1 ng
Benzo(b)fluorene	2 ng
Fluoranthene	25 ng

The fluoranthene value is high because of the lack of fine structure in the emission spectrum. The limiting factor in these measurements was the background caused by light scatter from the cellulose layer.

Quantitative determination of the separated PNA is based upon the peak area produced as the PNA spot is scanned. The instrumental parameters affecting quantitative scanning were studied and are discussed below.

Slits

Accurate reproducibility of all slit dimensions is absolutely necessary for quantitative TLC scanning. There are five variable slits in the Farrand-TLC Scanning system which was used. Four of these are the entrance and exit slits for both the excitation and emission monochromators and the fifth is the adaptor slit which defines the light beam image on the TLC plate. Fixed slits having 5, 10, and 20 nm band passes were available for each of the monochromator and 5 nm slits. All TLC scanning was done with a 10 nm slit at the entrance of the excitation monochromator and 5 nm slits at the three other positions. This combination gave the maximum sensitivity with sufficient resolution. Three fixed adaptor slits, having dimen-

sions 0.5 x 7.5, 1.5 x 13, and 2 x 14 nm were supplied with the instrument. The 1.5 x 13 mm adaptor slit was used for all TLC scanning since it provided the best compromise between sensitivity and resolution.

Wavelengths

Any wavelength between 220 and 700 nm could be selected with the excitation and emission monochromators with an accuracy of $\pm$ 2 nm. Qualitative scanning of developed plates to determine spot locations and subsequent R_B values was done with fixed excitation and emission wavelengths. For the analysis of a particular PNA in both standard and unknown samples, excitation and emission wavelengths corresponding to maximum adsorption and fluorescence bands respectively were chosen. The same wavelength settings were used when comparing the intensity of a spot from an unknown sample with that of a known amount of reference compound.

The use of monochromators in place of filters decreased the sensitivity of the scanner somewhat but had several distinct advantages. By the appropriate choice of excitation and emission wavelengths, it was sometimes possible to resolve two closely adjoining spots having different excitation or emission spectra. Using this method it was possible to combine several scans and obtain a composite picture of an entire chromatogram. An example of this process is shown in Figure 7. A separated mixture of the four reference hydrocarbons was scanned four times at differing excitation and emission wavelengths corresponding to the maximum response of the individual PNA. The bottom trace is a composite of the entire separation combined from the four individual scans.

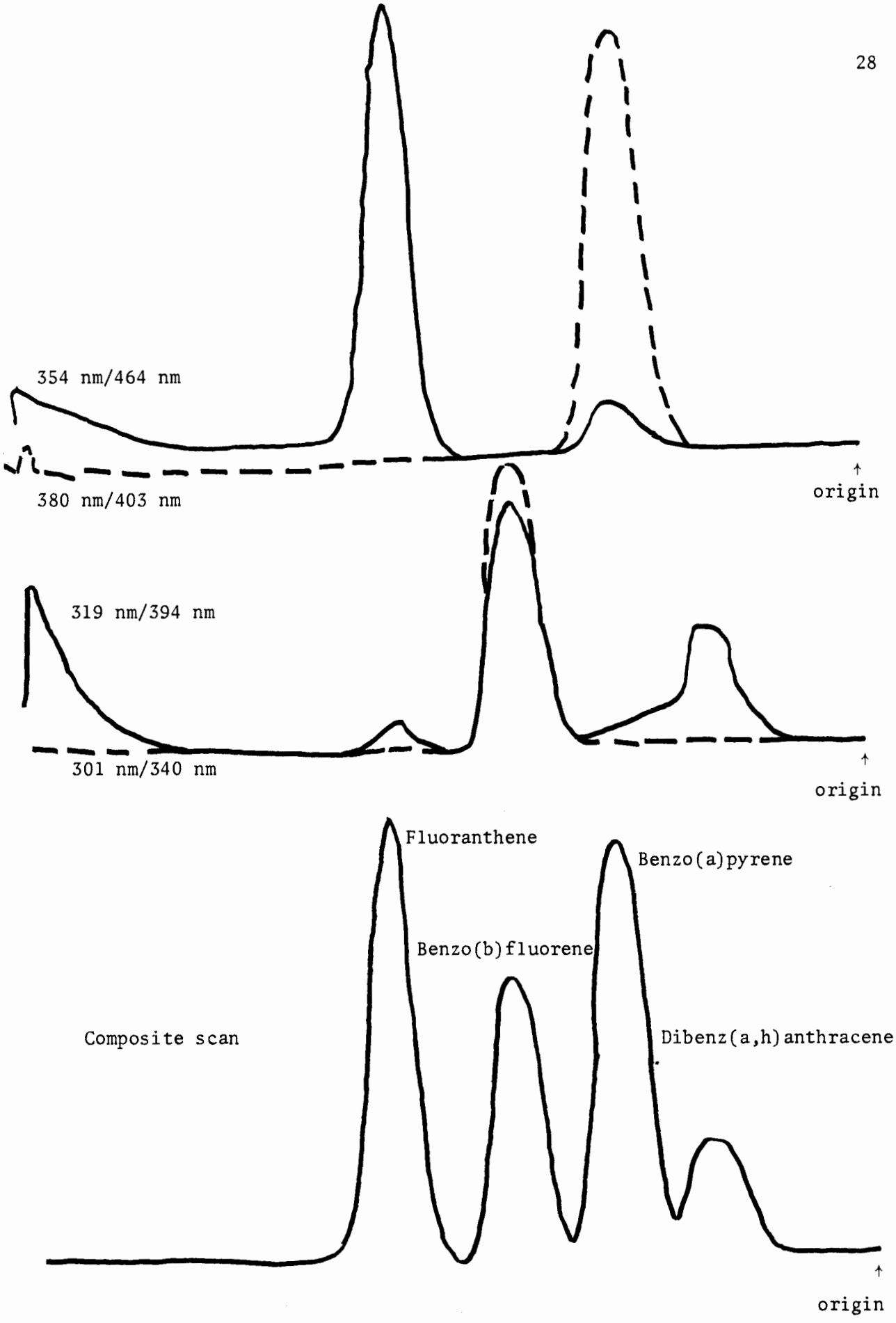


Figure 7. Formation of a composite TLC scan from individual TLC scans.

Distance from the adaptor slit to the adsorbent layer.

This parameter had to be held constant for all quantitative work. Variations in the height of the layer above the adaptor slit would have caused changes in the distances the excitation light travelled before striking the surface and also in the distance the reflected fluorescence traveled before entering the emission monochromator. Studies indicated a significant decrease in fluorescence intensity with increasing distance. On the other hand separate studies showed that the photodecomposition of spots increased with decreasing distance between the adaptor slit and the adsorbent layer. Since the effect on the fluorescence intensity was greater than that on spot photodecomposition, 1.5 mm was chosen as the standard distance for all further work.

Lateral position of the scanner

In order to insure reproducibility when scanning a single spot, the same area of the spot must be scanned each time. It was soon realized that the fluorescence response was susceptible to very small changes in the lateral position of the scanner. Deviations as small as 1 mm caused significant changes in fluorescence intensity. It was necessary, therefore, to determine the lateral position giving maximum response for each spot whenever quantitation was necessary.

Direction of Scanning

Location and identification of separated compounds is usually performed by scanning parallel to the direction of development. In this manner R_B values of the various spots may be calculated. For quantitative analysis, however, scanning either parallel to or perpendicular to the direction of development may be employed. Using single-beam instrumentation, perpendicular scanning is preferable when complete separation has been obtained because of the uniformity of the baseline.

In those cases where separations are not complete, parallel scanning is more advantageous and usually permits the estimation of unresolved peaks. Double-beam scanning, on the other hand, always employs parallel scanning in which the samples are spotted in alternate rows so that the reference beam can continuously sample the blank layer.

The scanner used in this work was a single-beam instrument. After location of individual compounds, quantitative scans were always run perpendicular to the direction of development.

Photodecomposition of PNA spots

Most PNA are highly susceptible to photodecomposition. Because photodecomposition could cause a significant error in quantitative measurements, several investigations were conducted to determine the extent of photodecomposition with the Farrand scanning system. A definite increase in photodecomposition as the plate was brought nearer to the adaptor slit was observed. The photodecomposition of 5 and 50 ng benzo(a)pyrene spots was also studied as a function of the number of times each spot was scanned. Each spot was scanned eight times with the intensity being maximized each time as quickly as possible. The areas under the respective peaks were plotted versus scan number and are shown in Figure 8. This data shows about 1% photodecomposition per scan for both the 5 and 50 ng spots. Light source intensity was shown to be constant by measurement of a quinine sulfate standard before and after the experiment.

Influence of time on fluorescence intensity.

Several authors have discussed the effect of time on the fluorescence intensity of separated compounds. It has been shown that the intensity is dependent upon the moisture content of the layer due

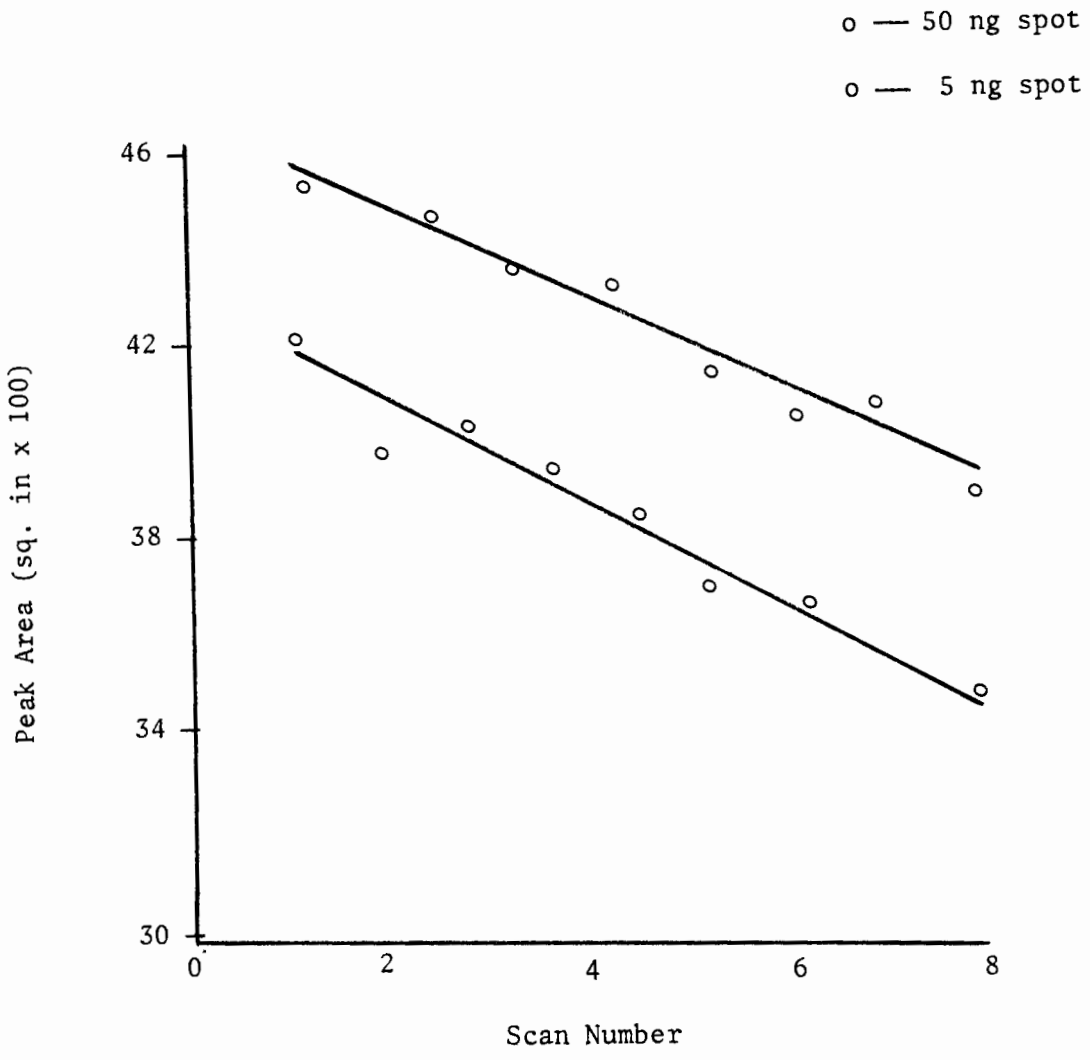


Figure 8. Photodecomposition of benzo(a)pyrene as a function of the number of scans.

to incomplete drying. Studies showed that there was no significant decrease in fluorescence intensity with time if the adsorbent layer was thoroughly dry.

Light Source Variation

The measured fluorescence of a spot is directly proportional to the light source intensity. For a single-beam system of the type used in this work, any changes in the intensity of excitation radiation product corresponding fluorescence changes. While the xenon-arc lamp was quite stable when allowed to warm up for about 30 minutes, incorporation of a reference system proved more desirable for performing accurate quantitative analysis. A 50 ng benzo(a)pyrene spot was used as a standard. The fluorescence emission was maximized before each scan, and correction was made to allow for the 10% photodecomposition accompanying each scan. The standard spot was scanned at regular intervals during the quantitative analysis of known and unknown PNA.

All of the above parameters must be considered when accurate quantitation is desired. Optimization of each parameter is highly desirable.

In order to accurately determine an unknown amount of compound by in situ fluorometry it was necessary to compare its fluorescence with that of known amounts of the same compound. This was usually done with the assistance of calibration curves in which the emitted fluorescence (area of the peak) was plotted as a function of the amount of compound applied to the layer. Measurement of peak areas was made with a planimeter. Although it was not absolutely necessary, it was advisable to work in a region where a straight line relationship between peak area and amount of sample is obtained.

Calibration curves were prepared for each of the reference compounds in the range of 5 to 50 ng. Typical curves for each of the reference PNA are shown in Figure 9. All of these curves were shown to be statistically linear by an analysis of variance. The range from 5 to 50 ng was therefore chosen as suitable for quantitative analysis of unknown amounts of each PNA.

Statistical analysis of the regression line equations obtained from four different plates showed that they were not identical at the 95% confidence limit. This meant that it was necessary to prepare a calibration curve on each plate. This was done in future unknown samples by spotting duplicate spots containing 5 and 50 ng of each of the reference PNA.

The reproducibility of scanning a single 50 ng benzo(a)pyrene spot was determined by scanning the spot 10 times with the maximization procedure being repeated between each scan. A relative standard deviation of 1.33% was obtained. The reproducibility of scanning six identical 50 ng benzo(a)pyrene spots was also studied in a similar manner. After the plate was developed and dried, the spots were scanned four times each in random order. The replicate data is shown in Table VII. The relative standard deviation for a single spot was 2.41% and that for six spot was 10.0%. This is the error for the entire measurement including spot application, variation of light source, decomposition, and instrumental error. The large difference between the errors for a single spot and all of the spots shows that the major portion of the error is associated with spot application, which is in agreement with previously published results.

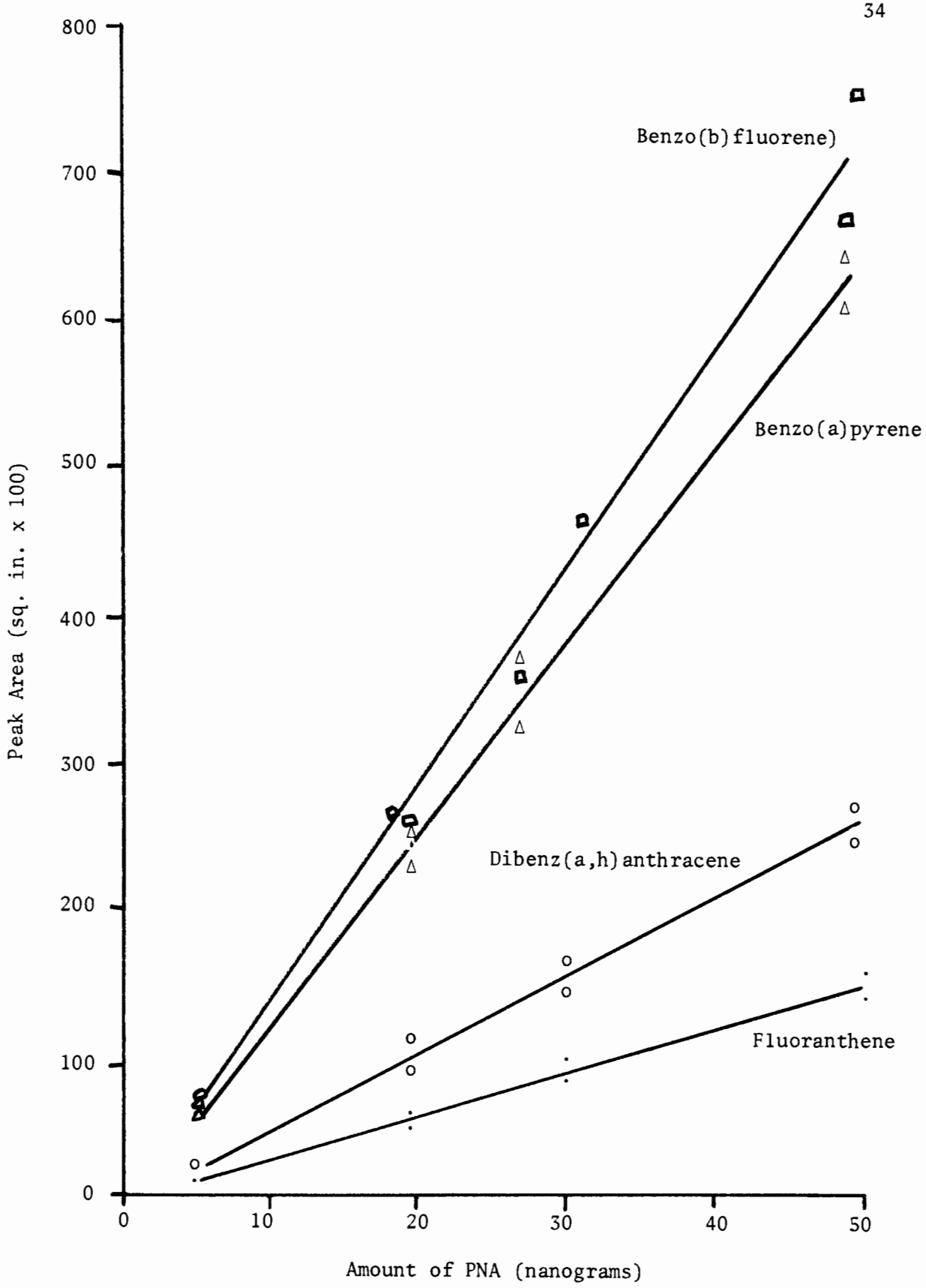


Figure 9. Calibration curves for four reference compounds.

Table VII.

Scanning Data for Reproducibility of Six Benzo(a)pyrene Spots

	1	2	3	4	5	6
Replicates	51	41	44	47	56	45
	53	42	47	47	54	45
	52	43	44	45	53	43
	52	41	44	47	55	43
Mean	52.0	41.8	44.8	46.5	54.5	44.0

Values are for peak areas expressed in square inches x 100

A series of experiments were carried out in order to determine the total recovery of the entire method for each of the reference compounds. In this way the amount of PNA lost because of incomplete extraction and surface adsorption could be calculated. Five analyses were carried out for each of the reference compounds using samples of known concentration. The results are shown in Table VIII.

In order to determine the actual amount of PNA present in an unknown water sample, the amount determined by analysis must be corrected for losses which result during the experimental procedure. A correction factor was calculated for each compound on the basis of its mean total recovery.

$$\text{Correction Factor} = \frac{100}{\text{Percent Recovery}}$$

The correction factors for the four reference compounds are listed below:

Dibenz(a,h)anthracene	1.34
Benzo(a)pyrene	1.32
Benzo(b)fluorene	1.29
Fluoranthene	1.14

To apply the correction factor to real samples the amount of PNA determined analytically is multiplied by the correction factor to obtain the actual amount present in the water sample. The use of this correction factor assumes that the total recovery of PNA dissolved in distilled water. Because very little is known about the physical state of PNA present in natural water, the validity of this assumption is uncertain.

Table VIII

Summary of Total Recovery Studies

	% Recoveries					Mean	Relative Standard Deviation
Dibenz(a,h)anthracene	77.0	68.5	67.5	78.0	81.0	74.4	11.1%
Benzo(a)pyrene	69.5	71.5	79.5	79.5	85.0	76.0	10.8%
Benzo(b)fluorene	70.0	75.0	84.0	86.0	72.0	77.4	9.3%
Fluoranthene	83.0	93.5	80.5	94.5	85.5	87.4	7.1%

ANALYSIS OF REAL SAMPLES

The developed method was used to analyze water from several New Hampshire rivers over a period of several years. Sampling locations on two of these rivers, the Oyster River and the Cocheco River, are shown in Figure 10. Sampling site A on the Oyster River was just above the U. S. Geological Survey Gaging Station in Lee. Three sites were chosen on the Cocheco River in Dover. Two of these were chosen above the city and one below the city in hopes of correlating PNA content with any possible pollution coming from the industries located in the city. Site B was located just upstream from the Washington Street bridge and the Dover sewage treatment plant below the city. Site C was located where County Farm Road crosses the river about 4 miles upstream from Dover. The final site D on the Cocheco River was just upstream from the Watson Road bridge approximately 2-1/2 miles above the city. The final sampling site E was located on the Winnipesaukee River in Tilton, New Hampshire. Water samples were taken below the bridge where Route 140 crosses the river. This site was chosen because of its proximity to a large asphalt plant located about 500 yards upstream.

Water samples were collected in 2 gallon polyethylene containers which had been thoroughly cleaned. The containers were washed with detergent, rinsed, washed several times with concentrated H_2SO_4, and finally rinsed thoroughly with distilled water. At the sampling site, each container was rinsed with river water before filling. Samples were taken just below the water surface being careful not to disturb the sediment on the river bottom. The water was transported quickly to the laboratory where the analysis was begun at once.

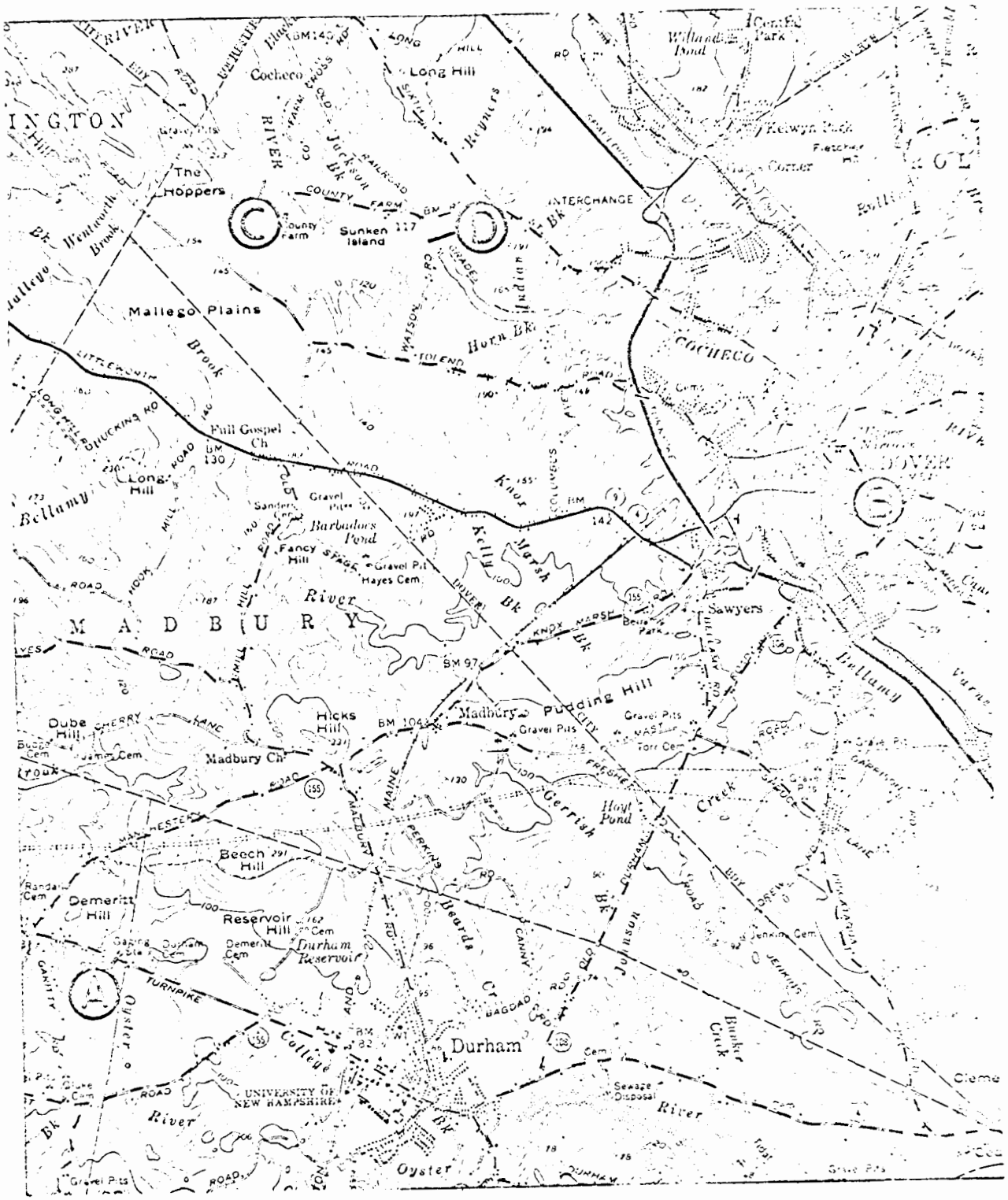


Figure 10. Location of Oyster River and Cocheco River sampling sites.

Because the method is concerned with nanogram amounts of substances, it is necessary to determine the contribution, if any, of contamination from sources other than the water sample.

Blank determinations were carried out periodically to determine any background contribution to the analytical method. Distilled water, whose purity had been checked fluorometrically, was either continuously or batch extracted, and the resulting extracts treated in the same way as unknown water samples. As in the analysis of unknown samples, the acid-washed extract was concentrated to dryness and the residue dissolved in 100 ul of ethanol. A 20 ul portion of this solution was applied to the TLC adsorbent so that 20% of the entire sample was analyzed.

Considering the smallest amount of each PNA producing a resolvable fluorescence spectrum, the minimum water sample concentrations which would be necessary to positively identify the four reference PNA by their fluorescence spectra are shown in Table IX.

None of the blank determinations using either continuous or batch extraction showed the presence of an identifiable amount of any PNA. Trace amounts of benzo(a)pyrene and benzo(b)fluorene were evident in several blanks using the continuous extractors but dibenz(a,h) anthracene and fluoranthene were never seen. Any error contributed by background contamination, therefore, was negligible compared with the error of the total method.

Ten river water samples were analyzed using the continuous extractors. One extraction per sample was performed for the first six samples while duplicates were performed in the final four analyses. These analyses were carried out in the period April 21, 1968 to May 10, 1970. Quantitative analysis was performed only on those spots which were positively identified by fluorescence spectra. A summary of the

Table IX

Identification Limits of Reference PNA in Blank Determinations

	Continuous Extraction	Batch Extraction
Dibenz(a,h)anthracene	5 ppt	8 ppt
Benzo(a)pyrene	1 ppt	2 ppt
Benzo(b)fluorene	2 ppt	3 ppt
Fluoranthene	25 ppt	42 ppt

Note: The detection limits for these compounds are approximately one-tenth of the identification limit values.

results of these extractions is presented in Table X. With the exception of fluoranthene in Sample No. 3, all concentrations were in the sub-part per billion range. Eight additional compounds, which were very likely PNA, were also detected in the water samples. All spots were characterized either by fluorescence spectra or the fluorescence excitation and emission wavelengths which gave maximum response. Spot H could not be recorded spectrophotometrically because its maximum fluorescence emission occured in the red wavelength region where the photomultiplier was insensitive.

Four river water samples were analyzed using the batch extractions from September 21, 1970 to October 1, 1970. Duplicate determinations were carried out for each sample using the technique of gently swirling the separatory funnel containing 3000 ml of water and 100 ml of pentane for 10 minutes. Each extract was washed with 72% H_2SO_4 and subsequently concentrated to dryness. Each residue was dissolved in 100 ul of ethanol and 20 ul applied to a cellulose sheet. No spots were visible in any of the chromatograms but fluorometric TLC scans did reveal some PNA. A summary of the results of these extractions is presented in Table XI. Although no PNA could be determined quantitatively, dibenz(a,h)anthracene and benzo(a)pyrene were characterized as being present. Several unidentified spots were also characterized as being identical with those obtained with continuous extraction.

Of the three rivers sampled, only samples from the Oyster River showed sufficient amounts of the four reference PNA to obtain fluorescence spectra. The concentration values determined for these four PNA agree with the results of Borneff, who has found total PNA concentrations of 0-10 ppt in ground water and 500-2600 ppt in German river water.

Table X

Summary of Continuously Extracted Samples

Sample	Identified Spots				Unidentified Spots	Sampling Site
	DiBahA	BaP	BbF	Fluor		
1	*	-	-	-	C,D,G	C
2	*	78	100	320	-	A
3	92	125	550	1000	-	A
4	*	-	*	330	H	A
5	-	-	155	-	B,F	A
6	*	150	240	-	A	A
7-B	*	*	*	*	-	A
7-C	-	*	*	*	-	A
8-B	-	*	-	-	-	B
8-C	-	-	-	-	-	B
9-B	-	*	-	-	E	D
9-C	-	*	-	-	E	D
10-B	-	-	-	-	-	E
10-C	-	-	-	-	-	E

Notes:

(1) *R_B values and maximum fluorescence wavelengths are correct but not enough compound is present for positive identification using fluorescence spectrum.

(2) Concentrations of identified spots are expressed as parts per trillion (ng/l) present in the original water sample.

Table XI

Summary of Batch Extracted Samples

Sample	Identified Spots				Unidentified Spots	Sampling Site
	DiBahA	BaP	BbF	Fluor		
B1	-	-	-	-	G	A
B2	*	*	-	-	C,D,G	C
B3	-	-	-	-	E	D
B4	-	-	-	-	-	E

* R_B values and maximum fluorescence wavelengths are correct but not enough compound is present for positive identification using fluorescence spectrum.

Fewer PNA could be characterized in water samples taken from the Cocheco River downstream from the city of Dover than in those taken upstream from Dover. This indicates that there is no significant contribution to the PNA content from sources within the city. The most probable sources of PNA within the city would have been suspended air particulate matter or industrial or sewage effluents. The fact that more PNA are found in the water above the city would indicate that these sources are contributing very little to the PNA content of the water.

No PNA could be detected in the two samples from the Winnipesaukee River. This site had been chosen because of its nearness to a large asphalt plant. PNA are known to be products of incomplete combustion of asphalt-type materials and it was felt that water taken from this location would contain large amounts of PNA. That there was indeed a great deal of extractable material in the water was apparent from the deep yellow color of the extract. This material was shown to be basic, however, by extraction with H_2SO_4.

When considering the possible sources of the PNA identified and characterized in this work, it is significant that the largest amount of PNA were found in the relatively unpolluted Oyster River. The Oyster River is small in comparison with the other two rivers sampled, and is fed by several small streams, themselves originating in swampy land. The banks of the river above the sampling site are generally steep, often rising 100 feet or more in a short distance. The large PNA concentrations found in the Oyster River suggest that the trace amounts of PNA present are due to natural phenomenon and not to environmental pollution.

SUMMARY OF THE METHOD

The purpose of this research was the development of a fairly rapid method for the quantitative analysis of PNA in natural water. A summary of the method is presented below.

1. Extract 3 liters of water with 100 ml of pentane for 10 minutes. Swirl the separatory funnel gently to avoid emulsion formation.

2. Concentrate the pentane extract to approximately 75 ml and extract one time with 25 ml of 72% H_2SO_4.

3. Concentrate the pentane layer to dryness and dissolve the residue in 100 ul of ethanol.

4. Apply 20 ul of the ethanol solution, as well as standard solutions containing 5 and 50 ng of each reference compound to a cellulose plate. Develop the plate in the dark to a height of 15 cm (approximately 4 hours). Dry the chromatogram in a stream of cool, dry air for 30 minutes.

5. Scan the plate parallel to the direction of development to locate the separated PNA and determine R_B values.

   Each separation should be scanned at a variety of fluorescence spectra of located spots wherever possible.

6. Scan perpendicular to the direction of development those spots which have been qualitatively identified. Compare the measured peak area with those obtained for 5 and 50 ng standard spots of the same compound.

The method is extremely sensitive and most PNA may be determined in the parts per trillion range. Extension of this range to lower limits can be accomplished by combining the extracts from several 3 liter water samples. The time required for a complete analysis using batch extraction is about 8 hours. If continuous extractions are necessary, an additional 24 hours is required.

Accurate quantitative measurements may be obtained for those PNA which can be completely separated on a cellulose plate developed with 50% aqueous DMF. Even in cases where individual compounds are not totally separated, the fluorescence of a single PNA can sometimes be measured by appropriate choice of excitation and emission wavelengths. Complex mixtures of PNA, such as those associated with air particulates samples, would require preliminary separation into simpler groups prior to TLC analysis. Several different TLC systems would be necessary to separate each group of PNA into individual compounds. Although the TLC scanning technique should be adaptable to these TLC systems, basic studies of the scanning parameters would be necessary.

The relative standard deviation of the entire method as shown in total recovery studies is between 10% and 15% for the four reference PNA. Since a significant percentage of this error is associated with manual TLC spot application, incorporation of an automatic TLC spotting device would reduce the relative standard deviation to less than 10%.

GAS CHROMATOGRAPHIC - MASS SPECTROMETRIC STUDIES

Phase II of this project has been to investigate the possibility that GC-MS might be useful as a qualitative tool for the analysis of aromatic hydrocarbons as well as other compounds of interest as possible pollutants, most notably organic acids. Two different methods, one primarily for neutral components and one for acidic components, have been developed. Both methods utilized a GC-MS approach. The following discussion will deal first with the GC-MS portion of both methods; the individual methods will then be discussed as to their unique aspects and the results obtained with each.

EXPERIMENTAL

The Gas Chromatograph - Mass Spectrometer

The gas chromatographic work in this study was done on a Perkin-Elmer, model 881, gas chromatograph equipped with a temperature programming unit. Twelve linear programming rates were available from 0.5° per minute to 48°C per minute. The model 881 contains dual injector ports and dual flame ionization detectors. The instrument can be operated in either the dual column or single column mode.

Independent heating controls for the injector block, column oven and detector are standard equipment. Since the detectors were located within the column's oven, the detectors maximum and minimum temperatures were dependent upon the column's operating temperature. With high temperature operation this was no problem. Near ambient temperatures, maintaining both the oven and detectors at a given temperature became difficult due to convection heating from the heated injector block.

Glass injector ports were used throughout this study. Pyrolytic decomposition was thus minimized in the injector block unit.

Specific operating conditions are shown in Table XII. These conditions were determined by an optimization procedure employing a 2n factorial design experiment.

The mass spectrometer used in this study was an Hitachi RMU-6E instrument. The entrance slit to the analyzer was set at a fixed slit width of 0.2 mm. A variable exit slit was used. Under normal operation, the exit slit was set at 0.5 mm. With this slit arrangement, a resolution of 700 (10% valley definition) was obtained when calculated using the mercury peak at m/e 200.

The instrument was equipped with a Honeywell, Model 1706, Oscillographic Visicorder. The visicorder was a direct-reading oscillograph designed to record four channels simultaneously. The recording frequency range was from DC to 5000 cycles, with a maximum writing speed of over 30,000 ins. per second. Eight paper speeds were available, 6.0 mm to 800 mm per second.

Using the visicorder, the reading of a mass spectrum from 0 to 300 mass units was performed in 3.0 seconds. A narrower mass range was used when the molecular weight of the compounds being examined was known. The scan speed and paper speed remained unchanged. This rate allowed taking several scans during the emergence of a single gas chromatographic peak.

The specific operating conditions for the mass spectrometer are shown in Table XIII.

Table XII

GC Operating Conditions

COLUMN	Length	50 feet	Diameter	0.02 in. i.d.
	Coating	SE-52	Concentration	Wall coated open tubular
	Support	None	Mesh	None
TEMPERATURE	Column: Init.	150°C	Final	190°C
	Rate	4°C/min	Det	225°C, Inj 225°C
CARRIER GAS	Helium		Rate	6 ml/min
	Pressure Inlet	20 psig	Outlet	Sub-ambient
	Hydrogen	13.5 psig	Air	40 psig
DETECTOR	FID			
	Sens	XI	Recorder Range	1 mv
SAMPLE			Size	1-3 ul
	Solvent	Benzene	Concentration	Variable

Table XIII

Mass Spectrometer Conditions

Connecting Line Temp	225°C	Setting	70
Inlet Box Temp	275°C	Setting	95
Ion Monitor Plate	Varies	Sensitivity	X1, X3
Recorder	1 mv		
$\frac{m}{e}$	450	Accel Voltage	
Repeller I	6 V	Grid I	-10 V
Repeller II	7 V		
Pressure*	4×10^{-6} Torrs		
Emission Regular			
Filament	3.4 A	Chamber	20 eV
Total	50 A	Target	80 V
Target	20 A	Grid	-20 V
		Repel I	6 V
		Repel II	7 V
Electron Multiplier			
EM	1500 Voltage	Sens	X10
G Heater	0.7 A		
Ch Heater	3.0 A		
Temp	250°C		
Visicorder Paper Speed	20 mm/sec		
Coil Current			
Start	$0m^A$	End	250 mA
Scan Speed	12	Time	3.0 sec.

* with GC line open.

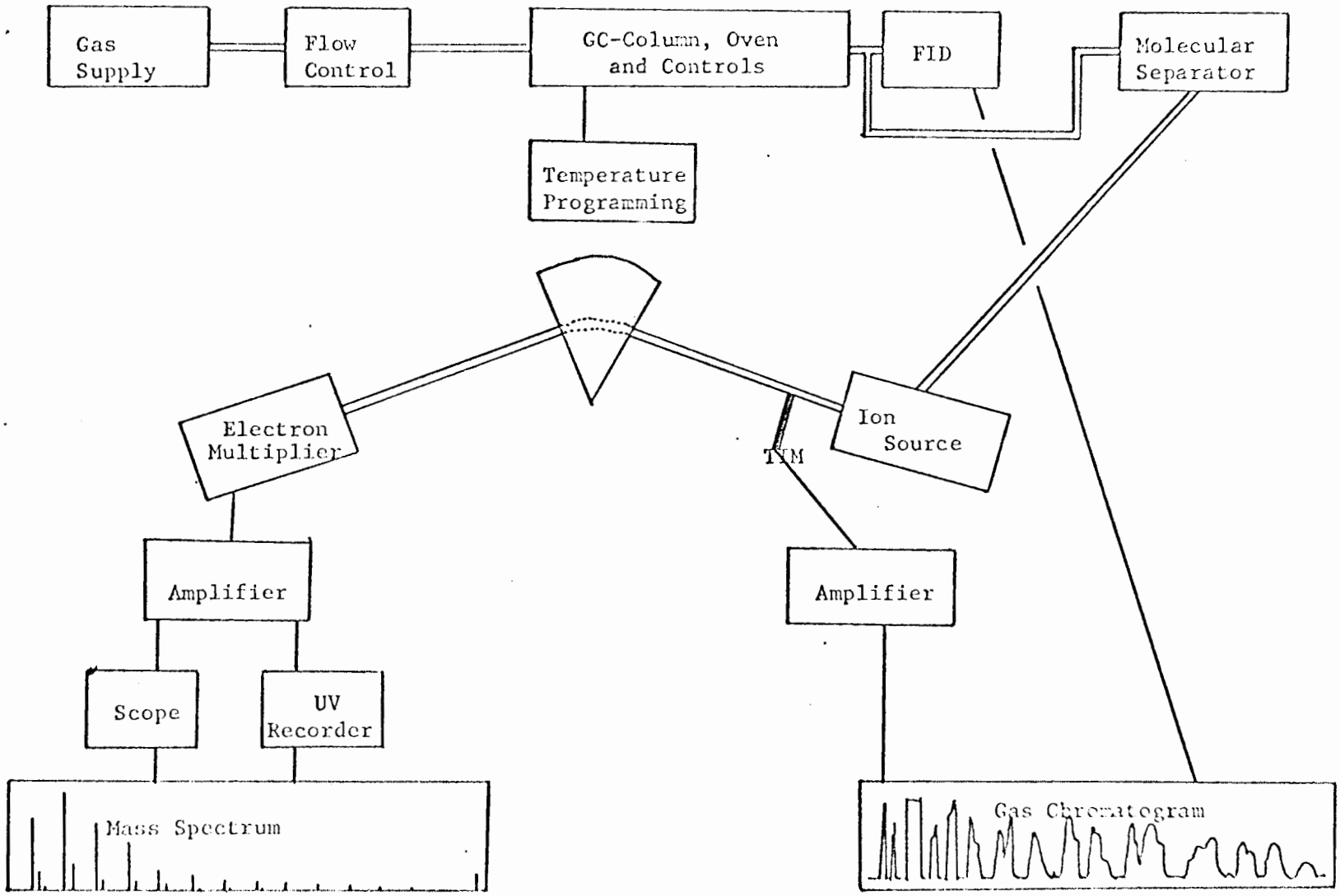
The gas chromatograph was connected to the mass spectrometer by means of a 0.01 in o.d. x 4.5-ft stainless steel capillary tubing. The capillary was enclosed in a 0.25 in o.d. x 4-ft aluminum tubing for added strength and protection. Two thermocouples were attached to the outside of the aluminum tubing. The thermocouples were spaced one foot from each end of the aluminum tubing. The temperature of the line was read on a Perkin Elmer TemperatureController by means of the two thermocouples. The aluminum tubing and thermocouples were wrapped with heating tape. The "Heat-by-the-Yard" tape, 0.5 in. wide x 8-ft long, was purchased from the Fisher Scientific Company. The temperature of the tape was controlled by a variable autotransformer.

The capillary line was connected to the exit pot of the receiving block in the gas chromatograph. The connection was made using a flow restrictor. Swagelok fittings and a Swagelok reducting union connected the restrictor to the capillary tubing. The restrictor was shortened, approximately two inches, to allow the connection to be made within the oven compartment of the chromatograph.

The mass spectrometer end of the capillary tubing was attached to a heated micro-capillary valve. The valve permitted separate operation of each instrument. The valve and capillary line were connected by means of 1/16 in. Swagelok fittings within the heated inlet box of the mass spectrometer. The valve was connected to the inlet side of an all-glass Watson-Biemann Separator by means of a 1/16 in. o.d. stainless steel capillary through a Kovar, metal to a glass seal.

A block diagram of the GC-MS system is shown in Figure 11.

Figure 11. Block Diagram of the GC-MS System



A number of studies related to the operation of the GC-MS system were then undertaken. First, the smallest detectable concentration which could be measured for the GC was determined for each of four polynuclear aromatic hydrocarbons. The conditions used were identical to those used when running unknowns using the GC-MS system except that the connection between the GC and the MS was closed. The results of this study are shown in Table XIV; the smallest detectable concentration was defined as that amount which gives a GC signal twice that of the noise level.

Fluoranthene was used as the standard to determine the fraction of the GC effluent going to the MS. The line to the mass spectrometer was closed and GC conditions were as reported in Table XII. An amount of Fluoranthene was injected into the GC sufficient to produce a detector response of around 80 to 90% full scale. Three identical injections were made. The line to the mass spectrometer was then opened. The conditions for the mass spectrometer and connecting line were as shown in Table XV. Three additional injections were made. The chromatograms were recorded as in the previous runs. The detector's response was lower with the mass spectrometer line opened. The difference in detector response represented the amount of fluoranthene going into the connecting line and to the mass spectrometer. The results indicated that under the experimental conditions used, 42% of the fluoranthene entered the connecting line. Of the 42% going to the mass spectrometer, part will be lost in the molecular separator. Therefore, not all of this amount reached the ionization chamber. No attempt was made to determine the amount lost in the separator unit. Also, no attempt was made to determine the amount entering the connecting line

Table XIV

Smallest Detectable Concentration Using FID

Compound	M.W.	R_t	Concentration ug
Fluoranthene	202	2.7 mins.	0.14
Benz(b)fluorene	216	3.5 mins.	0.31
Benz(a)anthracene	228	5.4 mins.	0.74
Benz(a)pyrene	252	14.5 mins.	2.00

One microliter of sample injected.

for other compounds. The amount will vary and depend greatly upon the carrier gas flow rate. The amount lost in the molecular separator will decrease with increasing molecular weight, since the rate of diffusion through the glass decreases with increasing molecular weight.

The standard solutions prepared for the determination of the GC detection limits were also used to determine the detection limit for the mass spectrometer. A sample size sufficient to give a GC peak was used at all times. The mass spectrum of the GC peak was taken at the peak's maximum. Several GC-MS runs were made. The concentration of the sample was decreased for each injection. By plotting intensity of parent ion vs concentration and calculating for approximately 42% of the fraction goes to the MS, it was possible to approximate the minimum concentration needed to obtain identifying spectra for the four compounds investigated. The GC-MS conditions were those presented earlier and shown in Tables XII and XIII. The results of this study are shown in Table XV.

One last general study was undertaken. It was to determine the approximate separation efficiency using the GC-MS system as opposed to using just the GC. It was assumed throughout that separation efficiency would be lost by coupling the MS to the GC; in fact these studies, using a series of n-alkanes from n-decane to n-octadecane showed that the GC-MS system had separation efficiencies approximately one-half those obtained with the GC system alone. Nevertheless, the separation efficiencies were sufficiently great to permit the effective utilization of the GC-MS for the purposes of interest to this study.

Table XV

Smallest Detectable Concentration Using MS

Compound	M.W.	Concentration* ug
Fluoranthene	202	0.07
Benz(b)fluorene	216	0.15
Benz(a)anthracene	228	0.37
Benz(a)pyrene	252	1.00

*Based on approximately 42% of injected concentration going to the mass spectrometer.

One microliter of sample injected.

Sample Preparation

For the analysis of PNA, duplicate 4.5 liter samples of fresh water were continuously extracted using n-pentane. Extraction was continued until the fluorescence level of the extract remained constant; this usually required about 24 hours. The pentane extract was then reduced to 25 ml, extracted with 72% sulfuric acid to remove basic compounds and taken down to dryness. The residue was dissolved in 25 microliters of redistilled benzene; one to three microliters of the benzene solution were injected into the GC-MS. The conditions used were as described earlier, see Tables XII and XIII; in addition the total ion monitor of the MS was adjusted such that approximately 20% of the ion beam was intercepted so that a continuous recording of peaks reaching the MS could be obtained. Mass spectra were recorded at the high point of the peak as determined from the total ion monitor.

For the analysis of organic acids, 5 liter samples of fresh water were collected, the pH adjusted to 2, filtered, and refiltered. Three and one-half liters of the five liter sample was passed through a 10 cm ion exchange column made of IRA-458 in the chloride form. The column was washed with 100 ml of water, pH 7, and then the acids were eluted with 100 ml of 1N NaOH. The effluent from the NaOH washing was reduced to approximately 10 ml; the pH was adjusted to 2; the acids were extracted with $CHCl_3$which was dried and evaporated to dryness. Since the chloroform did not extract all of the colored material, a second extract ion was performed with HCl saturated butanol. The butanol extract was evaporated to dryness; the organic material was taken up in acetone to separate it from the salt present; the acetone was evaporated off and the residue saved.

The acids from the chloroform and butanol extracts were methylated with 10% BCl_3MeOH; the methylated acids being extracted into chloroform which was dried, reduced in volume and used as such for analysis by GC-MS.

The ability of the procedure to in fact methylate the acids was checked. The acids examined were p-hydroxy benzoic acid, benzoic acid, 3-hydroxy-2-naphthoic acid, 1-naphthol, 2,4,6-trimethyl benzoic acid, caprylic acid, palmitic acid, stearic acid, o- and m-toluic acid. The dibasic and monobasic acids were easily methylated using the conditions specified in the experimental section. The extent of methylation was not determined. For the compounds examined, the phenolic group was not methylated. The carboxyl groups of 2,4,6-trimethyl benzoic acid, o-toluic acid and 3-hydroxy napthoic acid were not methylated with this reagent under the conditions used. This was predicted from the Victor Myer esterification law for sterically hindered positions. Methylation was checked by infrared and mass spectral data after precipitation from a suitable solvent system.

Water Collection Sites

All water samples analyzed were taken from rivers in the State of New Hampshire, primarily near the University of New Hampshire in Durham, New Hampshire. The specific locations are as follows:

Sample No. 1 was collected April 21, 1968 from the Cocheco River. The collection was made near the bridge on County Farm Road just above the County Farm. Labelled No. 1.

Sample No. 2 was collected September 30, 1968 from the Oyster River. The collection was made near the bridge on Route 4, west of Durham just below the Gaging Station. Labelled No. 2 and No. 3.

Sample No. 3 was collected November 7, 1968 from the Oyster River. The collection site was the same as Sample No. 2. Labelled No. 2 and No. 3.

Sample No. 4 was collected April 29, 1970 from the Cocheco River. The collection was made west of the bridge on Watson Road. It is labelled No. 4.

Sample No. 5 was collected November 5, 1970 from the Bellamy River. The collection was made near the bridge on Knox Marsh Road on the southeast side of the road. It is labelled No. 5.

In all cases approximately ten liters of water were collected. The water was transported to the laboratory for analysis immediately after collection.

Chemicals

All chemicals were of reagent grade and obtained from commercial sources.

Spectral Identification

Sample spectra were compared against reference spectra obtained from the API Catalog, Compilation of Mass Spectral Data, which uses the ten most intense peaks in a spectrum, literature sources and spectra obtained in our laboratory, both 20 eV and 70 eV. When possible more than one source was used because of the variation in spectra under various instrumental conditions. Good agreement between literature spectra and the 20 eV spectra obtained from the analysis existed in some cases. Most literature spectra were obtained at 70 eV, and it should be remembered in comparing these with 20 eV spectra that the lower ionization energy favors lower energy transitions.

When taken at 20 eV, the mass spectra for PNA have relatively simple fragmentation patterns, for this reason it was necessary to have additional qualitative information. The mass spectral patterns for methylanthracene and dimethylathracene consist of m/e (M+) and m/e (M+ - 15) for both compounds. Alone, these two peaks could not be used as positive identification. The R_t value for anthracene was available. By plotting R_t versus carbon number (the number of carbon atoms in the molecule) for anthracene, mono- and dimethyl anthracene, the points should fall on a single straight line. Figure 12 shows several such plots. The three points enclosed by circles give the R_t vs 14C, 15C and 16C which represents anthracene $C_{14}H_{10}$, methylanthracene $C_{15}H_{12}$, and dimethyl anthracene $C_{16}H_{14}$. A straight line is obtained through the center of each point. These two methods, GC-MS and R_t vs C plot, give strong evidence for the identification of these three compounds.

Additional plots are also presented, each plot strongly indicates that the individual point is a member of a homologous series. The points enclosed by a square represent a n-alkane series. The points enclosed by a triangle represent several ethyl ester series. The points enclosed by a diamond represents a series of methyl esters of n-acids.

Several plots for the same class of compounds were made when enough points were available. Although the slope from chromatogram to chromatogram changes, slightly, the lines are in the same general area on the graph for a given type of compound. This provides additional supporting evidence that a given type of compound was being analyzed.

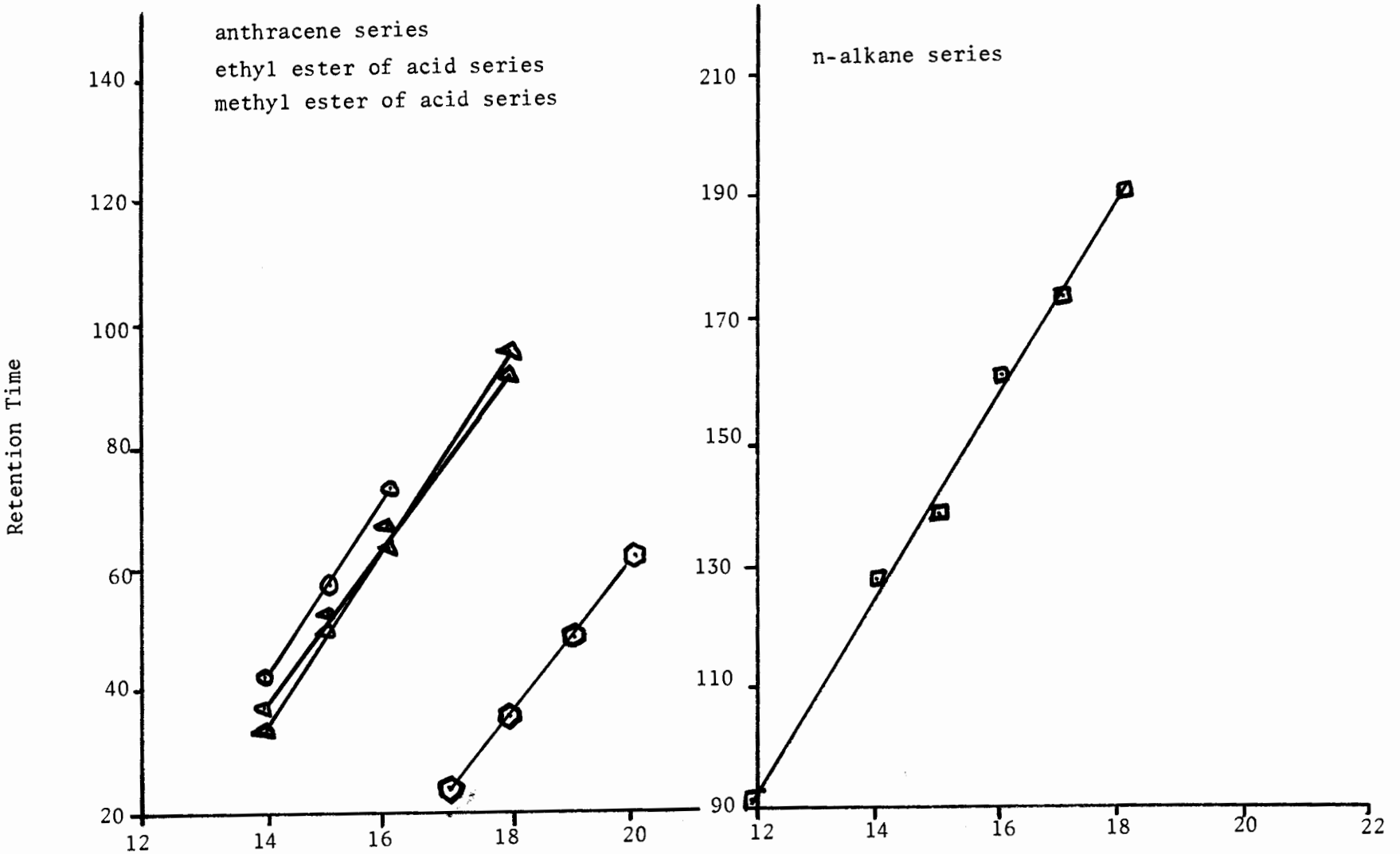


Figure 12. Plot Retention Time Versus Carbon Number.

Table XVII

Oyster River, Sample No. 2, September 30, 1968

Compound	Mol. Wt.	Figure No.	Ref. No.
n-Pentadecane	212	10	---
Unidentified	228	53	---
n-Nonadecane	268	13	---
n-Eicosane	282	14	AIP 705, 29M
Ethyl n-hexadecanoate	284	36	---
Fluoranthene	202	5	AIP 958
n-Heneicosane	296	15	---
n-Docosane	310	16	---
Methyl-Linoleate	294	31	117
n-Tricosane	338	17	AIP 1859, 1312
n-Tetracosane	348	18	AIP 575, 30M
n-Pentacosane	352	19	AIP 1313
Solvent impurity	---	--	---

Table XVIII

Oyster River, Sample No. 3, November 7, 1968

Compound	Mol. Wt.	R_t	Figure No.	Ref. No.
Ethyl n-tetradecanoate	256	34	34	---
Unidentified	228	40	53	---
Ethyl n-pentadecanoate	270	50	35	AIP 1006
n-Dibutyl phthalate	278	60	26	---
Ethyl n-hexadecanoate	284	64	36	---
Fluoranthene	202	73	5	AIP 958
Ethyl n-octadecanoate	312	95	38	---
n-Docosane	310	97	16	AIP 1311
Solvent Impurity	---	--	--	---

Table XVI

Cocheco River, Sample No. 1, April 21, 1968

Compound	Mol. Wt.	R_t	Figure No.	Ref. No.
Ethyl n-tetradecanoate	256	38	34	---
Anthracene	178	43	21	---
Ethyl n-pentadecanoate	270	53	35	---
Methyl anthracene	192	58	23	---
Ethyl n-hexadecanoate	284	67	36	---
Dimethyl anthracene	206	73	22	---
Fluoranthene	202	78	5	AIP 598
Ethyl n-octadecanoate	312	92	38	---
n-Tricosane	324	118	17	AIP 1859, 1312
n-Tetracosane	338	144	18	AIP 575, 30M
n-Pentacosane	352	185	19	AIP 1313
Solvent impurity	---	206	--	---
n-Hexacosane	366	244	20	AIP 1257

RESULTS

Summary of the Analysis of the Pentane Extractions

Four samples taken from New Hampshire rivers were qualitatively analyzed by a GC-MS system. Three classes of compounds were identified. Eleven different n-alkanes were identified ranging from n-pentadecane to n-hexacosane. Not all of these alkanes were identified in each river sample. The samples collected from the Cocheco River, April 1968 and April 1970, contained respectively four and five identified n-alkanes (see Tables XVI and XIX for specific compounds). In the 1968 sample, four high molecular weight n-alkanes ($C_{23}H_{78}$ through $C_{26}H_{54}$) were identified. The 1970 sample contained n-alkanes throughout the molecular weight range eluted under the GC conditions used for the analysis.

The September 1968 Oyster River Sample (see Table XVII) contained eight identified n-alkanes while the November 1968 (see Table XVIII) sample contained only one identified n-alkane. It is impossible to determine from the limited data a seasonal relationship or a clear indication as to differences in contamination level between the two rivers for this class of compounds.

Esters made up the second large group of compounds identified. Four different ethyl esters of n-alkyl acids were identified, at least one of these esters was found in each river sample. Two additional esters were identified; in the 1970 Cocheco River sample, n-dibutyl phthalate was identified and in the 1968 Oyster River sample, methyl linoleate was identified. Again, no seasonal relationship or difference in contamination level between rivers can be made.

Table XIX

Cocheco River, Sample No. 4, April 29, 1970

Compound	Mol. Wt.	R_t	Figure No.	Ref. No.
n-Heptadecane	240	24	11	AIP 1006
n-Octadecane	254	36	12	AIP 574
Unidentified	228	39	53	---
n-Nonadecane	268	49	13	AIP 592
n-Dibutyl phthalate	278	58	43	---
n-Eicosane	282	62	14	AIP 705,29M
Ethyl n-hexadecanoate	284	64	36	---
Dimethyl anthracene	206	69	22	---
Fluoranthene	202	78	5	AIP 598
n-Tricosane	324	133	17	AIP 1312

The final class of compounds to be identified was the polynuclear aromatic hydrocarbons. Four different PNA were identified. Fluoranthene was the only PNA to be found in all four samples. Anthracene, methyl- and dimethyl-anthracene were identified only in the 1968 Cocheco River sample. No correlation between rivers and seasons can be drawn.

The four PNA identified by the GC-MS were all highly fluorescent compounds and should be detectable by a TLC-fluorescent method. The inertness of n-alkanes and the fact that pentane, hexane, and other alkenes are recommended solvents for solution fluorescent methods, even high concentration of this class of compounds should not constitute an interference in fluorescence analysis. This may not be true for the esters. The presence of un saturation, the oxygen atoms and their possible interaction with active sites on the TLC adsorbent could cause this class of compound to constitute an interference in the TLC-fluorescent method. Additional studies will be required to clear up this point.

The banks or the tributaries of the Oyster River do not contain towns, cities or industrial complexes above the sampling site. The types of compounds identified from the Oyster River are not associated with agricultural fertilizers or pesticides. It must therefore be concluded that the compounds identified in this study originated largely from natural sources.

Summary of the Analyses of the Ion Exchange Extraction

Several elutions of the ion exchange column were made. This resulted in fractionation of the types of acids eluted from the resin. The first washing of the resin was made with 1N NaOH. The NaOH elution was further fractionated into the chloroform soluble acids and the n-butanol soluble acids.

These two acid fractions were then examined by a tandem GC-MS system. The chloroform fraction is presented in Table XX. The type of acids extracted with chloroform were not generally n-alkyl fatty acids. Only one such acid was identified, methyl n-tetradecanoate. This acid represents the highest molecular weight compound identified in the chloroform fraction. Only one additional acid ester was identified, methyl cinnamate. Seven unidentified compounds were also present. Because the molecular weights do not steadily increase with increasing R_t, it is assumed that more than one type of acid was present. A prominent feature in the fragmentation patterns for the unidentified acid esters was the m/e (M+ - 15 peak). This indicated that a favored fragmentation was the loss of a methyl group from the molecular ion. Since a m/e (M+ - 31) peak was not observed, it can be assumed that the methylated carboxyl group was not attached directly to an aromatic system, since the m/e (M+ - 31) would be expected in the fragmentation pattern for aromatic acid esters.

Table XXI presents the acid resulting from the n-butanol extraction. This fraction consists essentially of straight chain fatty acids. Nine such acid esters were identified, ranging from methyl n-decanoate to methyl n-nonadecanoate. Three unsaturated fatty acid esters were also identified: methyl octadecantrienoate, methyl octadecandienoate and methyl octadecenoate. Two aromatic acid esters were identified: n-dibutyl phthalate and dimethyl terephthalate. The n-butanol fraction differs significantly from the chloroform fraction.

Table XX

Bellamy River, Sample No. 5, Chloroform Extract

	Mol. Wt.	R_t	Figure No.	Ref. No.
Unidentified	98	14	48	---
Unidentified	138	--	49	---
Unidentified	154	48	44	---
Unidentified	138	55	50	---
Unidentified	178	63	46	---
Unidentified	160	109	51	---
Methyl cinnamate	162	115	40	---
Unidentified	178	---	45	---
Unidentified	200	188	41	---
Methyl n-tetradecanoate	242	202	25	---

Table XXI

Bellamy River, Sample No. 5, n-Butanol Extract

Compound	Mol. Wt.	R_t	Figure No.	Ref. No.
Unidentified	138	30	49	---
Unidentified	140	39	none given	---
Unidentified	170	49	none given	---
Methyl n-decanoate	186	53	39	117
Unidentified	154	67	none given	---
Unidentified	156	73	none given	---
Dimethyl phthalate	194	86	47	---
Methyl n-dodecanoate	214	93	24	117
Unidentified	212	97	none given	---
Unidentified	226	105	none given	---
Methyl n-tetradecanoate	242	128	25	117
Methyl n-pentadecanoate	256	139	30	117
n-Dibutyl phthalate	278	152	43	117
Methyl n-hexadecanoate	270	161	26	117
Methyl n-heptadecanoate	284	174	27	117

Table XXI (continued)

Compound	Mol. Wt.	R_t	Figure No.	Ref. No.
Methyl octadecantrienoate	292	185	32	117
Methyl octadecandienoate	294	185	31	117
Methyl octadecenoate	296	185	33	117
Methyl n-octadecanoate	298	191	28	117
Methyl n-nonadecanoate	312	---	29	---
Unidentified	314	---	52	---

It can be seen from Tables XX and XXI that several of the lower molecular weight compounds were incompletely extracted by chloroform. It can also be seen that the chloroform solvent, often used for the extraction of acids, did not adequately extract the high molecular weight n-alkyl acids. The bulk of this class of compounds appeared in the n-butanol extract. Yet the conditions of the chloroform extract, acidic pH and high salt concentration, implies good conditions for high extraction efficiency of acids. It is this type of qualitative information which is needed to understand and devise better procedures as required in the quantitative analysis of complex pollution analysis.

N-dibutyl phthalate was the only strange "ester" among the esters identified. Its presence can be explained as follows. The phthalic acid isomers were extracted by n-butanol. The solvent, n-butanol, was removed by evaporation with a small amount of heat required. During the heating process the ortho-phthalic acid was converted to the anhydride, which reacted with the alcohol to give the n-dibutyl ester of phthalic acid. The meta- and para-phthalic acids are not so easily converted to the anhydride. It was for this reason that the n-butyl ester of o-phthalic acid was detected and the methyl esters of the m- and p-isomers of phthalic acid were detected.

Mass spectra were also obtained for three components which were eluted as a single symmetrical peak from the GC. The components were identified as the methyl ester of linolenic acid, which has three double bonds each separated by a methylene group, methyl esters of linolic acid, which has two double bonds separated by a single methylene group and the methyl ester of oleic acid, which has only one double bond. By taking sequential mass spectra as the peak eluted into the

spectrometer, the mass spectrum for each component could be obtained. The earliest spectra were characteristic of the methyl ester of linolenic acid. Positive identification for these three acid esters was made using this technique of successive scanning. Several other intense chromatographic peaks were examined by this technique. These peaks appeared to be single component peaks.