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RECENT ADVANCES IN THE GAS AND LIQUID CHROMATOGRAPHY OF FLUORESCENT COMPOUNDS

I. A DIRECT GAS-PHASE ISOLATION AND INJECTION SYSTEM FOR THE ANALYSIS OF POLYNUCLEAR ARENES IN AIR PARTICULATES BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

A gas-phase isolation and injection system has been devised that completely eliminates the need for solvents in the preparation of polynuclear arene-containing air particulate samples for analysis by gas–liquid chromatography. The entire package consists of a remote, preparative multi-sample stripper unit, a gas-phase injector mounted on a gas chromatograph, and a gas-phase spectrophotofluorometric detector. Advantages of the gas-phase method over traditional solvent extraction procedures include short per-sample preparation and handling times, and, since sample size can be relatively large, good sensitivity.

INTRODUCTION

Air particulates often contain polynuclear arenes (PNAs), some of which are carcinogenic. The particulates are usually collected for analysis by trapping them on fiber glass filters which then must be extracted for isolation of PNAs[1,2].

The PNAs are bound firmly to the particulate matter. Consequently, conventional Soxhlet extractions are incomplete, irreproducible, and time consuming. Also, expensive glassware, organic solvents, and manpower are required. Liquid-phase extraction using an ultrasonorator to dislodge the PNAs is much more efficient than Soxhlet extraction[3]. However, the extract must be concentrated and usually only a small aliquot of the concentrate is injected into the gas chromatograph, which results in reduced sensitivity.

Gas-phase extraction is more efficient. PNAs contained in particulate matter which has been trapped in fiber glass air sampling filters can be removed by a stream of nitrogen at 300°. The compounds are trapped in a cold column which contains the same packing that will subsequently be used for gas–liquid chromatography (GLC). The cold column is then attached to a gas chromatograph, heated, and the compounds separated on the main column. They are measured as they elute with a gas-phase

spectrophotofluorometric (SPF) detector. Because of the specificity of this detector, prior cleanup of the sample is not required and compounds which are not separated on the column frequently can be resolved optically. The electron capture detector (ECD), which is frequently used for the analysis of PNAs, is virtually inoperable under these conditions.

This combined gas stripping–gas-phase SPF detection system eliminates the need for nanograde organic solvents, standard laboratory glassware, liquid extraction, sample cleanup, and concentration. It also greatly reduces the number of man hours required per sample.

EXPERIMENTAL

Stripping equipment

Stripping is carried out in 9 mm O.D. × 160 mm borosilicate glass tubes which are contained in each of six 9.5-mm holes bored through a stripper block which consists of a 3 in. × 3 in. aluminum cylinder (Fig. 1). The upper end of each tube is connected to a gas manifold with standard metal tube compression fittings through a 3/8 in. to 1/4 in. reducing union. Two silicone rubber O-rings are used to replace the

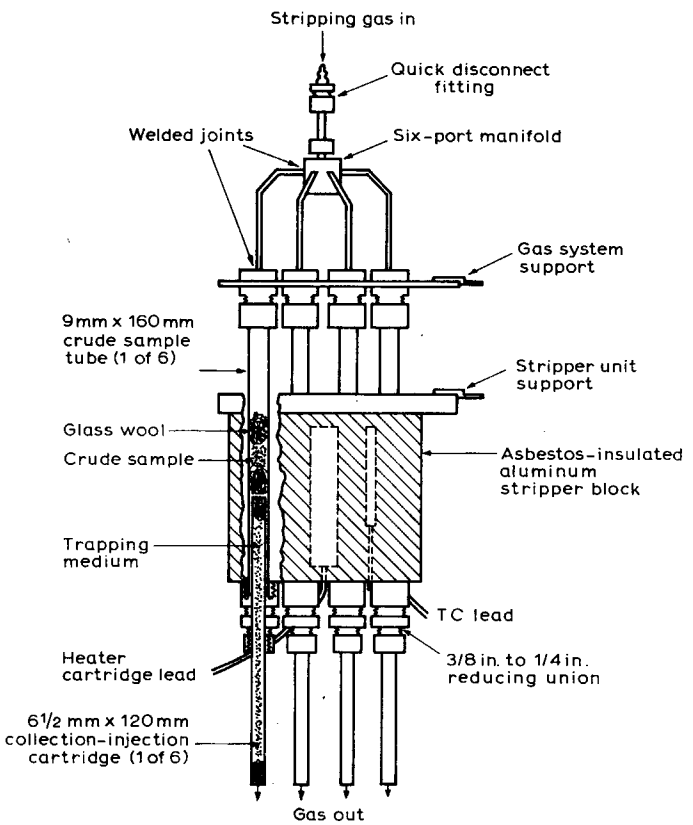


Fig. 1. Stripper unit and detail of crude sample and injection tubes. TC lead = thermocouple lead.

front ferrule of the fitting and the back ferrule is reversed. The lower end of each stripping tube is connected to a 6.5 mm O.D. × 120 mm borosilicate glass precolumn with a similar fitting except that it is bored to a diameter of 1/4 in. to accept the column. The aluminum block and tubes are heated with a 250-W cartridge heater which is centrally located and held at a preset temperature with a temperature controller.

Stripping procedure

Hi-Vol air filter mats (any other filter which is not degraded by heat can be used) containing air particulates are ground to about 20 mesh in a Wiley mill. The samples (about 100 mg) are then loosely packed into the stripper tubes and held in place with tightly fitting plugs of glass wool. The tubes are inserted in the aluminum block and the upper ends attached to a gas manifold which in turn is connected to a cylinder containing compressed nitrogen. The lower ends of the tubes are attached to precolumns which are packed with the same stationary phase used for chromatography. The aluminum block is heated to 300° and nitrogen is passed through the system at a rate of about 50 ml/min. Stripping is carried out overnight. Due to the long stripping time variations in flow-rates between the tubes are unimportant. The precolumns are ready for analysis immediately after cooling and removing them from the stripper.

Gas-phase injector

The injector consists of a chamber-and-valve combination with appropriate fittings to attach it to the gas chromatograph and for insertion of the precolumn sample tubes (Fig. 2). The back of the chamber is made from a 1/2-in.-thick aluminum block bored to hold a 300-W cartridge heater and thermocouple and the cover is made from 1/8-in.-thick aluminum channel. Rapid heating is possible using this heater. The entire chamber is insulated with 1/2-in.-thick asbestos pads. The precolumn fittings are ordinary 1/4-in. compression fittings welded to 1/16-in. stainless-steel tubing which connects with the injector valve. About 4 ft. of this 1/16-in. tubing on the upstream side of the loop containing a precolumn is coiled around the glass tube. The 8-port injector valve (Valco, CV-8-HT) is encased in a machined aluminum heater assembly which is maintained at 300° at all times with a 100-W cartridge heater. This temperature is maintained with an auxiliary controller available as an option with the 5700 Series Hewlett-Packard gas chromatograph. The valve is attached to the rear port of the standard heated dual injection ports of the instrument. A modification has been made so that a continuous stack of aluminum plates or blocks reaches from the valve enclosure to the injection port heater. This may be seen in Fig. 2, which is a drawing of the entire injector.

Injection procedure

A precolumn is inserted in the injector chamber and sealed with compression tube fittings and O-rings as before. The direction of gas flow through the tube is maintained as it was during stripping. The heated 8-port valve is kept in the position that forces carrier gas through a short closed loop and then into the analytical column at all times except during an actual injection. Thus when the cover of the injector chamber is put in place and purge gas introduced at the proper inlet, the column segment is swept free of oxygen and made ready for injection (Fig. 2)*. Following a brief

* Appropriate quick connecting tube fittings on the nitrogen gas line, the stripper manifold, and the purge line inlet permit convenient dual usage of this single gas source.

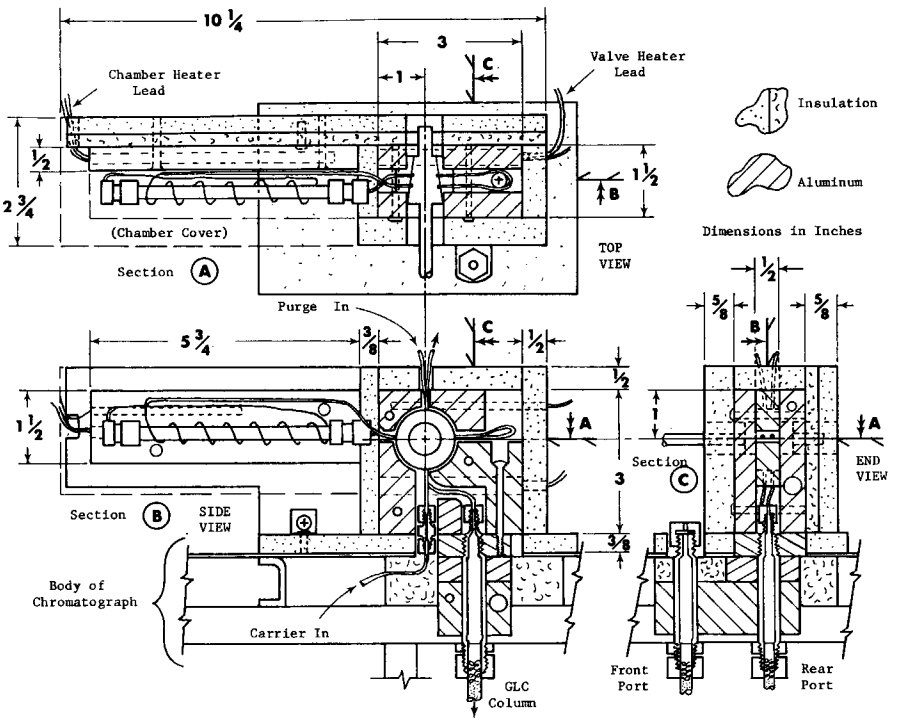


Fig. 2. Details of gas-phase injector.

purge period (about 5 min) the valve is turned to direct carrier gas through the sample tube and rapid heating of the chamber is begun. The 300-W cartridge heater imbedded in the aluminum back of the injector oven is operated at full 120 V until a thermocouple installed in the same block has reached 450°. An automatic power shutoff then occurs and the temperature begins to drop. This high temperature is not reached within the glass tube itself and is only an empirically determined value which corresponds to a maximum temperature of about 320° (Fig. 3) inside the sample cartridge.

The valve is left in this position for an additional 5 min and then returned to the standby purge position. The time required for heating is approximately 10 min so that the overall start-to-finish injection period lasts 15 min. The cover of the chamber is then removed and the spent sample cartridge and fittings are rapidly cooled with an air blower. Chromatography continues during this cooling step and also during the insertion and purging of the next sample.

Chromatography

A 6 ft. × 1/4 in. O.D. glass column packed with 100–110 mesh Anakrom A coated with 5% by weight of Dexsil 300 GC® is used. A 3 ft. × 1/4 in. O.D. glass column packed with 80–100 mesh Chromosorb G HP coated with 10% OV-1 is substituted when shorter retention times are desired. The former column is operated at 270° and the latter at 250°. Instrumental conditions routinely used are: injection

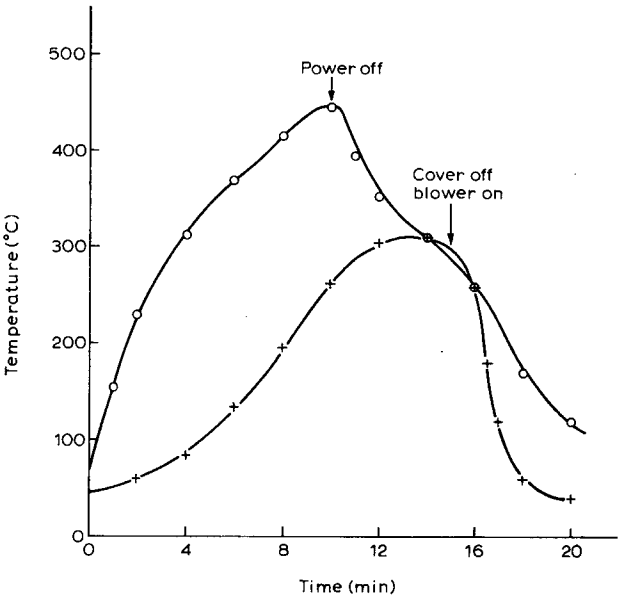


Fig. 3. Injector temperature changes *vs.* time during gas-phase injection sequence. ○, Aluminum block interior; +, sample tube interior.

port, ECD, transfer line, and SPF detector cell block temperature, 300°; column oven temperature, 250–270°. The carrier gas flow-rate is 100 ml/min. Half of the gas passes through the gas-phase injector valve and half through the remaining unaltered injection port after which both streams are combined prior to entering the analytical column.

Detection

The PNAs are detected as they elute from the column, first with an ECD and then with a gas-phase SPF detector[4,5].

The ECD is part of a standard Hewlett-Packard (Model No. 5713A) gas chromatograph which is reported to be linear over a wide range[6]. The detector is pulsed and requires a mixed carrier gas (argon–methane, 90:10) for best performance.

The ECD and SPF detectors are connected by a 2 ft. × 1/16 in. O.D. × 0.03 in. I.D. stainless-steel tube. The transfer line is heated by a 25-ft. piece of asbestos-insulated resistance wire (yellow, 24 Ga., chromel "A" wire, 1.6 Ω/ft.) and insulated with woven tubular fiber glass cloth and asbestos tape. The temperature of the transfer line is monitored with a thermocouple and kept at 300° with a variable voltage transformer. Standard compression fittings are used to seal the transfer lines to both the exit of the ECD and the base of the SPF cell compartment. The compartment contains a 3 in. × 1/4 in. × 1/4 in. quartz flow cell sealed with O-rings and compression fittings to the transfer line terminal fitting (Fig. 5). Carrier gas containing the eluted compounds flows through the cell from bottom to top and exits to an exhaust duct opening just above the compartment. The quartz cell holder is heated with a 100-W cartridge heater and kept at 300° by a variable transformer.

TABLE I

GAS CHROMATOGRAPHY OF POLYNUCLEAR AROMATIC HYDROCARBONS

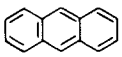
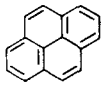
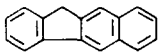
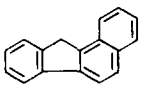
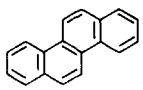
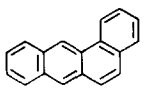
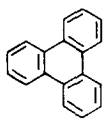
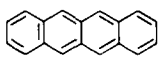
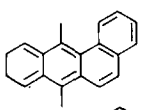
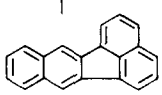
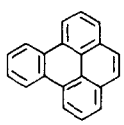
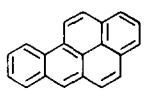
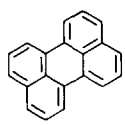
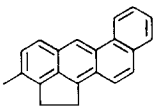
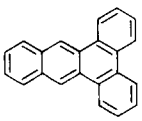
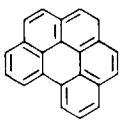
Compound	*Structure*	*Relative retention time**
Anthracene		0.066
Pyrene		0.15
Benzo[*b*]fluorene		0.19
Benzo[*a*]fluorene		0.30
Benzo[*c*]phenanthrene (Chrysene)		0.30
Benz[*a*]anthracene		0.36
Triphenylene		0.37
Benz[*b*]anthracene (Tetracene)		0.40
7,12-Dimethylbenz[*a*]anthracene		0.46
Benzo[*k*]fluoranthene		0.81
Benzo[*e*]pyrene		0.98
Benzo[*a*]pyrene		1.00
Perylene		1.09

TABLE I *(continued)*

Compound	*Structure*	*Relative retention time**
3-Methylcholanthrene		1.41
Dibenz[*a*,*c*]anthracene		2.1
Benzo[*ghi*]perylene		2.4
Coronene		5.6

* Relative to benzo[*a*]pyrene = 1.00.

The SPF detector is an Aminco-Bowman spectrophotofluorometer modified to function as a gas-phase instrument. The excitation and emission monochromators are set at appropriate wavelengths for best average response for the PNAs of interest (Table I) and the slits at the various optical windows set for either maximum sensitivity (wide) or maximum selectivity (narrow). The output of a 150-W Xenon lamp focussed through an optical off-axis condensing mirror system provides sensitivity of

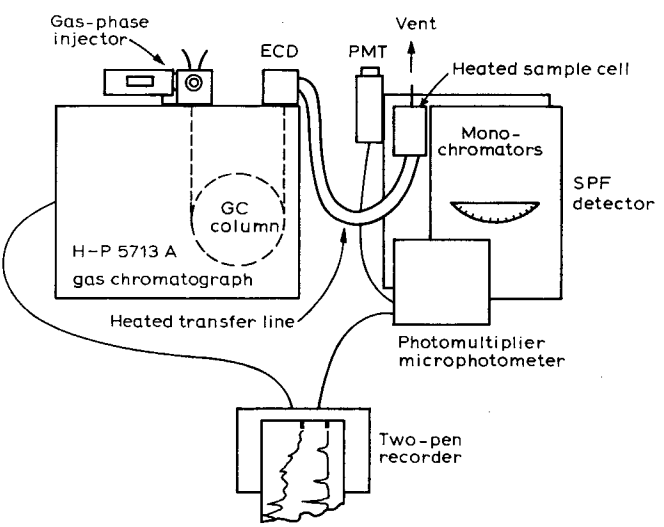


Fig. 4. Gas-phase injector–gas chromatograph–spectrophotofluorometric detector combination. PMT = Photomultiplier tube.

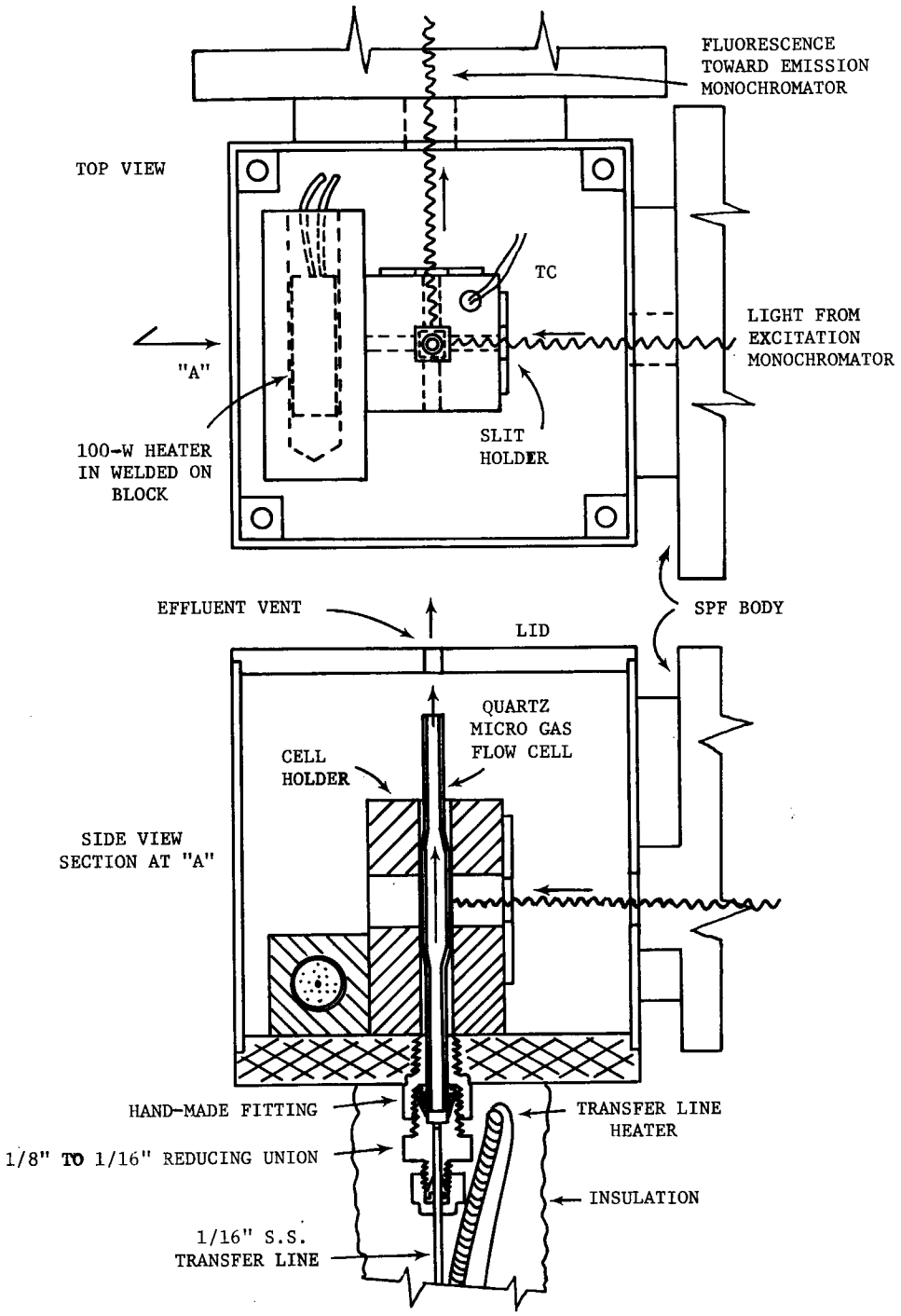


Fig. 5. Gas-phase SPF sample compartment.

3 to 100 ng ($S/N = 3$) depending on compound, wavelength settings, and quality of focussing adjustment. The overall arrangement of the GLC–SPF combination is depicted in Fig. 4 and details of the SPF cell are shown in Fig. 5.

DISCUSSION

Quantitation

In order to evaluate the reproducibility and recovery of the total GLC–SPF system, a Hewlett-Packard Model 3380-A Reporting Integrator was used to monitor the SPF detector responses for a variety of samples. A standard solution containing pyrene, chrysene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, and perylene was made up in hexane at the concentrations of 102, 77, 7.4, 64, and 22 ng/μl, respectively. This solution was used in three different ways to determine the reproducibility of normal syringe-septum injection, injection directly on the precolumn for gas-phase injection, and injection on blank filter mat for stripping, trapping in the precolumn, and then gas-phase injection.

A set of wavelengths was chosen that gave best average response for pyrene, chrysene, benzo[*k*]fluoranthene, and benzo[*a*]pyrene. Perylene was not chromatographically resolved from benzo[*a*]pyrene with the 3-ft. OV-17 column employed, but its response was only about 2% of the benzo[*a*]pyrene response so it was neglected in the calculations. The wavelengths chosen were 290 nm for excitation and 420 nm for emission.

TABLE II

GAS-PHASE FLUORESCENCE WAVELENGTH MAXIMA FOR SELECTED PNAs

Values in parentheses are for pentane solutions taken from E. Sawicki[7].

PNA	*Excitation λ*	*Emission λ*
Fluorene	272 (300)	330 (321)
Chrysene	262 (264)	386 (381)
Benzo[*k*]fluoranthene	298 (302)	424 (400)
Benzo[*e*]pyrene	280 (339)	394 (400)
Benzo[*a*]pyrene	290 (300)	411 (413)
Perylene	244 (430)	433 (438)

A series of eleven sequential septum-syringe injections of the standard solution was made by two persons using two different 10-μl syringes. The data obtained with the Hewlett-Packard integrator are shown in Table IIIA, where σ is the standard deviation and V the coefficient of variation. Since the number of samples was small, the calculation for σ was based on $\sqrt{[\Sigma(X - \bar{X})^2/(N - 1)]}$. Comparable data for standard samples injected onto the precolumn and then gas-phase injected were obtained. Each such injection was compared by area counts to syringe-septum injections made immediately before and after to obtain recoveries of each component of the mixture. These data are shown in Table IIIB for nine such bracketed recovery measurements. The same kind of data were obtained for standards injected into filter mat which was stripped overnight, the compounds collected in precolumns, and then gas-phase injected. Bracketed syringe-septum and syringe-precolumn standards were used to determine recoveries for five such samples as shown in Table IIIC.

TABLE III

REPRODUCIBILITY DATA

	Pyrene	*Chrysene*	*Benzo[k]fluoranthene*	*Benzo[a]pyrene*
A. Syringe-septum				
Integrator counts $\bar{X}$	305	126	450	2281
σ	7.03	3.16	13.4	79.0
V	2.30	2.51	2.98	3.46
B. Syringe-precolumn				
Per cent recovery $\bar{X}$	85.3	83.9	86.7	82.1
σ	2.74	6.75	8.09	6.06
V	3.21	8.04	9.33	7.38
C. Stripped precolumn				
Per cent recovery $\bar{X}$	86.9	92.8	90.1	83.4
σ	5.58	7.52	14.8	5.64
V	6.42	8.10	16.4	6.76

Stripping

Air particulates containing PNAs may vary considerably in composition, size distribution and amounts of PNAs present. As a result of these and other considerations the efficiency of removal of organic compounds from particulate matter by any method may likewise vary. Of particular importance is the fact that PNAs are strongly absorbed on the exposed and hidden surfaces of particulates, especially soot, and thus not as easily removed as from inert surfaces[8]. This holds true for either liquid or gas-phase isolation procedures and accounts for variations noted in results of PNA analysis.

In an attempt to minimize these variations and eliminate the problems inherent in solvent methods of PNA fraction isolation, we developed a gas-phase procedure for this step.

To achieve this, the prototype unit described previously was built for simultaneous stripping of six samples. Apparatus could easily be constructed for stripping many more samples than this.

This stripping process removes PNAs from unused filter mats in about 10 h. Hence, stripping was carried out overnight to insure complete removal of PNAs and minimize the effects of differences in flow-rate between tubes which might cause unequal recovery.

Checks were made to be certain that PNAs did not escape the cool collection tubes on prolonged stripping and it was found that when these tubes were packed with coated solid support only small losses occurred. Only small amounts of PNAs vaporized from sample tubes could be trapped in precolumns packed with uncoated Anakrom A.

Injection

The gas-phase injector is essentially a part of the chromatograph injection port and column oven with the capability of independent flow and temperature control. When a collection tube is inserted into the injector chamber it becomes a part of the analytical column separated from it by a few inches of heated transfer line and valve. This arrangement does not significantly affect resolution or peak shape.

The preliminary purging step to remove air speeds the recovery of the ECD and in any case is desirable from the standpoint of prolonging column life. Passage of air through a hot column often leads to rapid degradation of the liquid phase by oxidation. Runs made without purging did not show any interference by air with the SPF detector, which had been one of the main concerns, but the ECD performance was poorer.

Rapid heating of the precolumn suffices to quickly volatilize the condensed PNAs into the carrier gas stream flowing through the tube where chromatographic resolution begins to occur. This continues in the rest of the analytical column after the PNAs have passed through the valve and connecting tubing.

The time for a single injection is virtually the same as that required to heat the injector oven from 80° to 450°. This is usually 10 min, with slight variations, depending on the frequency of injection. The valve directing carrier gas through the precolumn is maintained in this position for an additional 5 min to insure complete removal of slowly moving compounds. For example, if the retention time of the slowest moving compound is 30 min in the 6-ft. column then the residence time in a 6-in. precolumn would only be $2\frac{1}{2}$ min.

Chromatography

GC separation of injected PNAs is the primary method of resolving the components of the mixtures. This yields satisfactory resolution for all but a few combinations of PNAs. Relative retention data shown in Table I, while not meant to be complete, give an indication of some of the more difficult separations. Benzo[*a*]- and benzo[*e*]pyrene have similar retentions. Although other workers have reported resolution of this refractory pair[9,10], we have been unable to satisfactorily resolve them chromatographically.

One of the septum injection ports was left intact on the instrument for use in checking chromatographic conditions. Syringe injection of PNA standards into this port, of course, yields chromatograms more quickly than can be obtained by gas-phase injection.

Better comparisons can be made of standards *vs.* samples, by injecting directly on the packing of the precolumn and using this for gas-phase injection. Such samples provide chromatograms whose peak profiles and retention times are more directly comparable to stripped standards which require overnight preparation.

The ECD is sufficiently sensitive for measurement of PNA standards by GLC. Its sensitivity is a drawback, however, in the gas-phase injection method described here. Impurities in the sample swamp the ECD and it does not recover until near the end of the run.

The SPF detector, on the other hand, is remarkable for its inability to distinguish between syringe-injected standards and gas-phase-injected samples. The SPF detector is thus the most important feature of the instrument package contributing to full utilization of the gas-phase injector. It does not sense the presence of non-fluorescent impurities, normal changes in gas flow, pressure, or composition. The gas-phase SPF detector is essentially independent of anything except the presence or absence of gaseous fluorescent materials in the optical path in the sample cell. This selectivity to fluorescent compounds is further enhanced by the fact that response is obtained only when the excitation and emission wavelengths of the fluorescent

compound are matched with the settings on the two monochromators of the fluorometer.

This property of selectivity can be further exploited to provide resolution of compounds not separated chromatographically. For example, the excitation and emission wavelengths that yield the greatest fluorescent response for benzo[*a*]pyrene and perylene are quite different. The signal due to perylene in a 1:1 mixture of the two is negligible when the optimal values for benzo[*a*]pyrene are used. This capability of selective fluorescence detection does not eliminate all the difficulties presented by poor chromatographic separations but it minimizes such problems in many cases.

The relatively high carrier gas flow-rate used in the system is required for successful operation of the SPF detector. Low flow-rates lead to broadened skewed peaks that are not duplicated by the ECD signal. This appears to result from a need to rapidly sweep the quartz flow cell with effluent gases to prevent the buildup and consequent overlap of previously resolved components. The high flow-rate reduces ECD sensitivity, but is nonetheless important for best overall instrument performance.

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