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DETECTION OF GAS CHROMATOGRAPHY ELUATES BY SIMULTANEOUS ABSORBANCE AND FLUORESCENCE MEASUREMENTS

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SUMMARY

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

INTRODUCTION

Polynuclear aromatic substances (PNAs) and their derivatives are spread widely throughout the environment by industrial effluents, combustion of fossil fuels by vehicles and power plants, forest and agricultural fires, and refuse burning[1]. Many of these compounds are known mutagens and suspected carcinogens[2]. Accordingly, much effort has been directed toward developing reliable analytical methods for measuring PNAs.

Analyses for PNAs in environmental or biological samples are often complicated by interfering compounds[3]. Even highly selective spectroscopic techniques such as Shpol'skii fluorescence require some sample clean-up[4], and less selective methods may require extensive sample pretreatment[5]. Instruments combining chromatographic resolution with sufficiently selective detectors may allow for less sample pretreatment in PNA determinations. Thus, uncertainties in measurement due to interfering compounds may be avoided.

PNA analyses have been described which use liquid chromatography (LC) with UV-absorbance[6], fluorescence[7], or photoionization[8] detection. Detectors used for gas chromatographic (GC) analysis of PNAs include flame ionization[9], electron capture[10], mass spectrometry (MS)[11], UV absorbance[12], pyrolysis–chemiluminescence[13], and fluorescence[14].

We have previously described PNA measurements by a gas phase fluorescence

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TABLE I

GAS PHASE ABSORBANCE–FLUORESCENCE DETECTION OF SELECTED AROMATIC COMPOUNDS

LR = Linear dynamic range; LOD = limits of detection; S = sensitivity.

Compound (% purity)	t_s *(min)*	*LR (ng)*	*Absorbance*		*Fluorescence*	
			LOD (ng)	*S (absorbance units* ng^{-1}*)*	*LOD (ng)*	*S* (μg^{-1})*
Naphthalene (98)	0.5	50–500	43	$0.36 \cdot 10^{-5}$	ND	–
Azulene (99)	0.8	45–450	8	$2.05 \cdot 10^{-5}$	ND	–
Acenaphthylene (95)	1.4	180–1800	97	$0.18 \cdot 10^{-5}$	ND	–
Dibenzofuran (98)	1.8	50–550	46	$0.39 \cdot 10^{-5}$	238	5.1
2,3,5-Trimethylnaphthalene	2.3	100–1000	64	$0.30 \cdot 10^{-5}$	172	7.7
Fluorene (98)	2.3	45–450	23	$0.82 \cdot 10^{-5}$	220	1.7
Dibenzothiophene (95)	4.1	49–500	42	$0.54 \cdot 10^{-5}$	ND	–
Acridine	4.4	45–450	41	$0.57 \cdot 10^{-5}$	ND	–
Phenanthridine (98)	4.9	55–550	30	$0.82 \cdot 10^{-5}$	ND	–
Xanthone (99)	5.2	98–1000	72	$0.34 \cdot 10^{-5}$	192	5.0
2-Methylanthracene (97)	5.8	45–450	26	$1.02 \cdot 10^{-5}$	85	15.7
9-Chlorophenanthrene (98)	6.5	147–1500	24	$1.14 \cdot 10^{-5}$	91	2.2
2-Nitrofluorene (98)	7.7	90–900	72	$0.42 \cdot 10^{-5}$	ND	–
9-Bromoanthracene (95)	7.9	220–2250	61	$0.50 \cdot 10^{-5}$	ND	–
Pyrene (99)	8.0	49–500	10	$3.05 \cdot 10^{-5}$	42	26.6
9-Nitroanthracene (97)	8.3	96–1000	31	$1.00 \cdot 10^{-5}$	ND	–
2,3-Benzofluorene	9.1	25–2500	42	$0.79 \cdot 10^{-5}$	9	72.9
9-Phenylanthracene (98)	10.8	50–500	37	$0.97 \cdot 10^{-5}$	130	6.3
Triphenylene (98)	11.1	45–450	32	$1.16 \cdot 10^{-5}$	125	10.3
Benzo[*a*]pyrene (98)	14.9	25–250	34	$1.34 \cdot 10^{-5}$	16	60.9
Benzo[*e*]pyrene (99)	15.0	49–500	41	$1.09 \cdot 10^{-5}$	62	16.6
1,3H-Dibenzo[*a,i*]carbazole (98)	19.1	250–2500	60	$0.89 \cdot 10^{-5}$	22	71.7
1,2,3,4-Dibenzanthracene (97)	20.1	45–450	21	$2.66 \cdot 10^{-5}$	15	60.1
1,2,5,6-Dibenzanthracene (97)	20.7	49–500	36	$1.59 \cdot 10^{-5}$	42	28.8

* Sensitivity for fluorescence calculated according to a method described in ref. 1.

detector for GC[14]. A standard flame photometric detector was modified using a UV light source, a monochromator, and a quartz fiber optics bundle. In the system described here, the detector cell includes two important modifications: (a) a second fiber optics bundle, filter, and photomultiplier tube (PMT) in a linear configuration to monitor UV absorbance, and (b) bandpass filters to select both the excitation and emission wavelength bands for fluorescence.

The additional selectivity offered by this system for multimode detection may be helpful in environmental, toxicologic and other analyses where interferents often co-elute with analytes. Using two detection modes with different selectivities helps to avoid effects of co-eluting interferents. For example, GC–MS has been used in conjunction with LC–UV spectrometry[15] and LC–UV-absorbance systems have been used which concurrently monitor at two different wavelengths[5]. Gas phase absorbance measurements also extends the range of detectable compounds, as compared to gas phase fluorescence.

EXPERIMENTAL

Reagents

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

Five PNA solutions were prepared and diluted to determine sensitivities, ranges of linearity, and limits of detection. The composition of these solutions were as follows, with concentrations indicated in parentheses ($\mu g/\mu l$).

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

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

Solution C. Dibenzofuran (0.11), xanthone (0.20), 9-chlorophenanthrene (0.30), 9-bromoanthracene (0.45), 2,3-benzofluorene (0.025), benzo[*a*]pyrene (0.05), 1,3H-dibenzo[*a,i*]carbazole (0.05), 9-phenylanthracene (0.10).

Apparatus

A schematic representation of the system is shown in Fig. 1. GC separations were performed on a Tracor Instruments Model 565 gas chromatograph. Eluates flowed directly from the column into an aluminum detection cell heated to 250°C with a "Z" flow path (see Fig. 2). The 1.6-cm horizontal section of the flow path in the cell was lined with a Suprasil quartz tube, 4 mm I.D. Light from a deuterium source of a Tracor Model 970A detector was passed via a condensing mirror through a 260-nm bandpass filter into a 1 m × 3 mm Welch Allyn high-temperature quartz fiber optics bundle. The other tip of the fiber optics bundle, fitted into the detector cell with o-rings and PTFE sleeves, illuminated the horizontal flow path. Light emitted from the fiber optics bundle has *ca.* 60° dispersion, resulting in decreasing illumination intensity along the eluent path.

Fluorescence measurements were made through a window perpendicular to

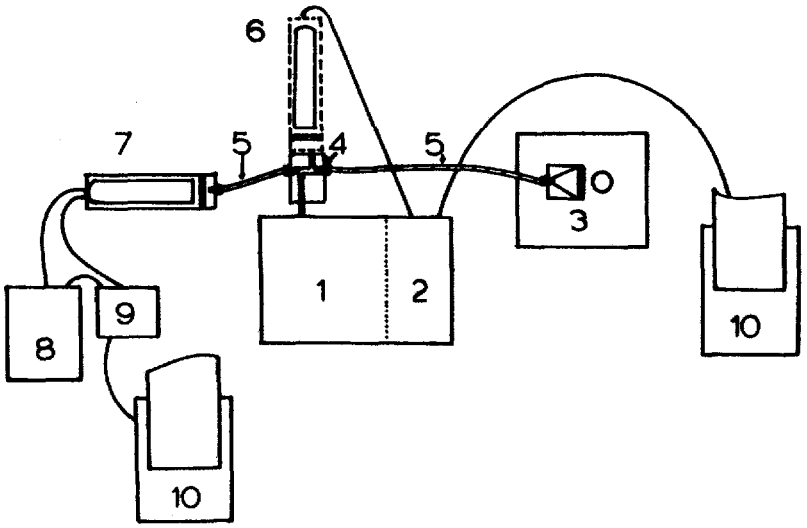

Fig. 1. Schematic representation of simultaneous absorbance–fluorescence GC detection systems; 1 = GC; 2 = signal amplifier; 3 = $^2H_2$ lamp, 260-nm bandpass filter; 4 = heated detector cell; 5 = fiber optics bundles; 6 = 340-nm longpass filter, 380-nm bandpass filter, and fluorescence PMT; 7 = 240-nm bandpass filter and absorbance PMT; 8 = high-voltage power supply; 9 = absorbance signal modifier; 10 = digital integrators.

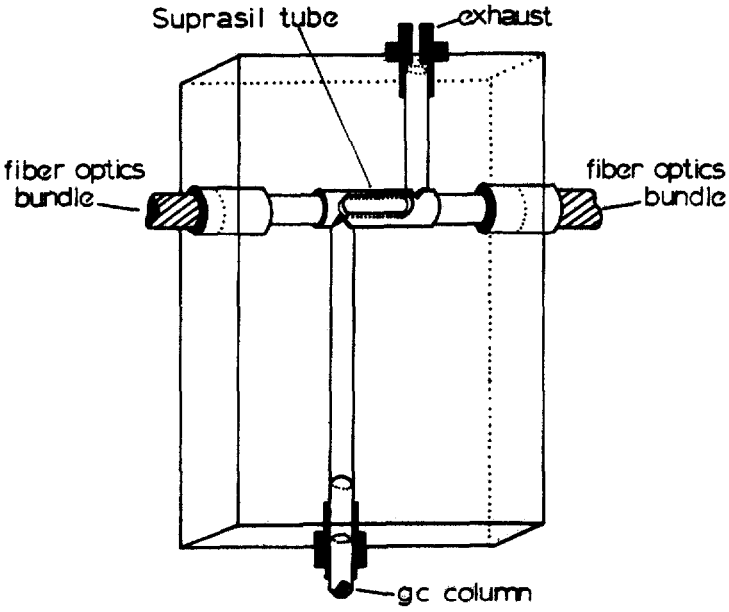

Fig. 2. Heated, flow-through detector cell with volume of 200 μl.

the illuminated path. Fluorescent light from this window passed through 340-nm long-pass and 380-nm bandpass filters (see Fig. 3) and was detected by a head-on PMT. The distance from the side window to the photocathode of the fluorescence PMT was approximately 15 cm. Fluorescence chromatograms were recorded on a Hewlett-Packard Model 3390A digital integrator.

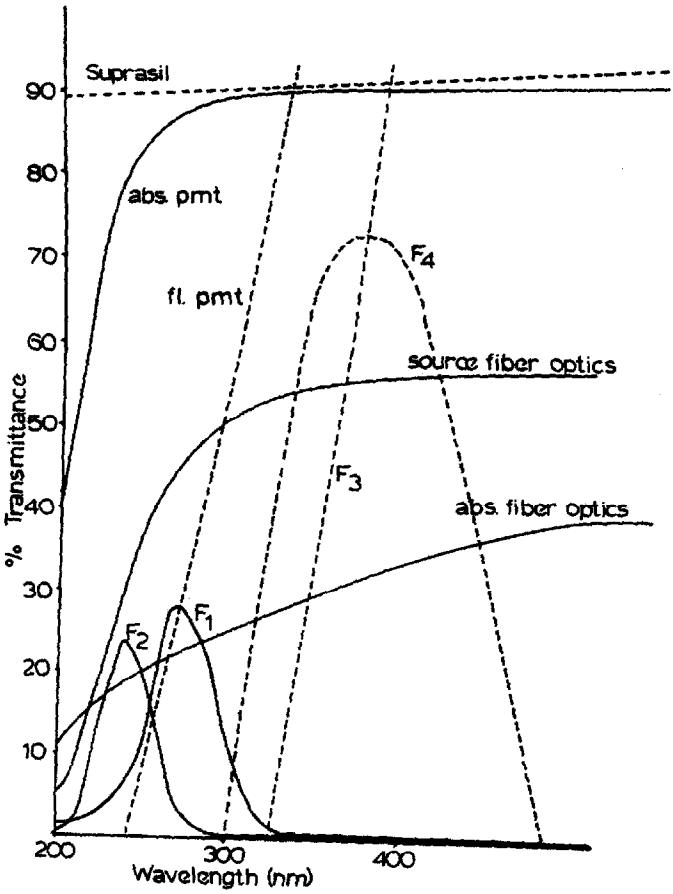

Fig. 3. Transmittance (%) *vs.* wavelength for optical components of absorbance–fluorescence GC detector: F_1 = 260-nm bandpass filter (source); F_2 = 240-nm bandpass filter (absorbance); F_3 = 340-nm longpass filter (fluorescence); F_4 = 380-nm bandpass filter (fluorescence); fl. pmt = PMT window (fluorescence); abs. pmt = PMT window (absorbance).

Light for absorbance measurements was collected in a linear configuration by a 250 mm × 1 mm Maxlight high-temperature fused-silica fiber optics bundle. Light issued from the end of this fiber optics bundle passed through a 240-nm bandpass filter (see Fig. 3) to a Hamamatsu R1464 head-on PMT. The distance from the fiber optics bundle tip to the photocathode of this PMT was approximately 5 mm. The signal from the PMT was modified by a Jfet operational amplifier current-to-voltage convertor with a 0.1 sec time-constant, followed by an Analog Devices logarithmic amplifier to convert transmittance to absorbance. The absorbance signal was then

attenuated and offset by an operational amplifier voltage follower with appropriate gain and voltage offsets. Chromatograms of absorbance were recorded on the integrator from a Hewlett-Packard 5880 gas chromatograph using an external input board.

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

Procedure

Injections (5 μl) were made for serial dilutions of each solution. A temperature program was used with an initial temperature of 150°C for 2 min, and then changed at 10°C/min to a final temperature of 250°C for 20 min. N_2 carrier gas was used with a flow-rate of 30 ml/min.

RESULTS AND DISCUSSION

The heated flow-through detector cell (Fig. 2) accepted GC eluates directly from the column, eliminating chromatographic band broadening and adsorptive losses in transfer lines from the GC to the spectrophotometric detectors. In addition, the design featured a small detector volume of 200 μl to maintain chromatographic resolution of peaks. This design resulted in a viewed volume for the fluorescence measurement of approximately one-thirtieth that of a previously described fluorescence detector[14]. However, strong fluorescent signals were obtained for many multiring PNAs.

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

Optical filters transmitted wavebands which are characteristic for PNAs. Fig. 3 shows properties of the various optical components of the system. The transmitted excitation and emission wavebands for the fluorescence measurement have maxima at 259 nm and 390 nm, respectively. The transmitted light for the absorbance measurement passes through both 260-nm and 240-nm bandpass filters to narrow the waveband yielding a maximum transmittance of about 255 nm and a bandwidth at half height of approximately 20 nm. The arrangement of these two filters with the shorter waveband filter following the longer waveband filter eliminates fluorescent light detection by the absorbance PMT.

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

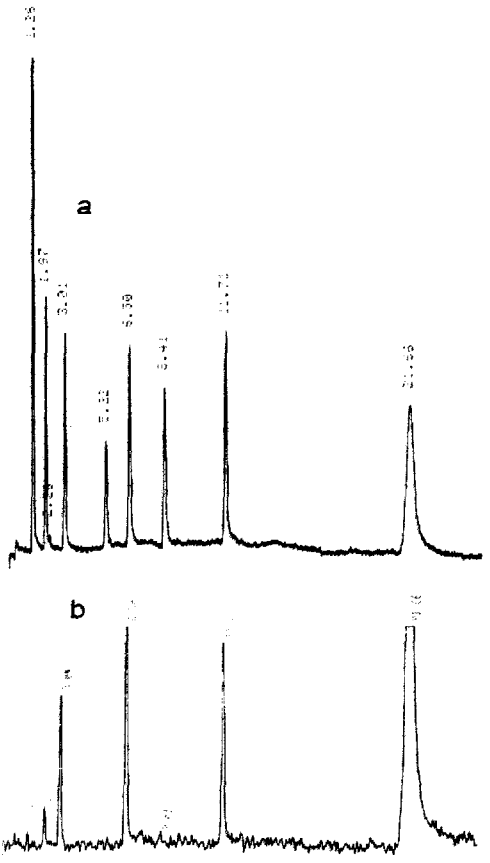

Fig. 4. Chromatograms of PNA solution produced by simultaneous (a) absorbance and (b) fluorescence detection: 1.26 min = 450 ng azulene; 1.97 min = 1800 ng acenaphthylene; 3.01 min = 450 ng fluorene; 5.22 min = 450 ng acridine; 6.50 min = 450 ng 2-methylanthracene; 8.41 min = 900 ng 2-nitrofluorene;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

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

All aromatic compounds tested were detected by absorbance and sensitivities for these substances did not differ greatly for multiringed systems or for aromatics with a variety of functional groups. Not all of the aromatic compounds were detected by fluorescence. The best sensitivities were obtained for systems containing three or more fused rings. In addition, certain functional groups, such as the nitro group, decreased the fluorescence signal, even for high-molecular-weight multiring aromatics.

Selective detection of low-molecular-weight aromatic compounds by absorbance but not fluorescence was demonstrated by analysis of an extract from a cigarette filter (Fig. 5). Many substances were detected by absorbance, but not by fluorescence.

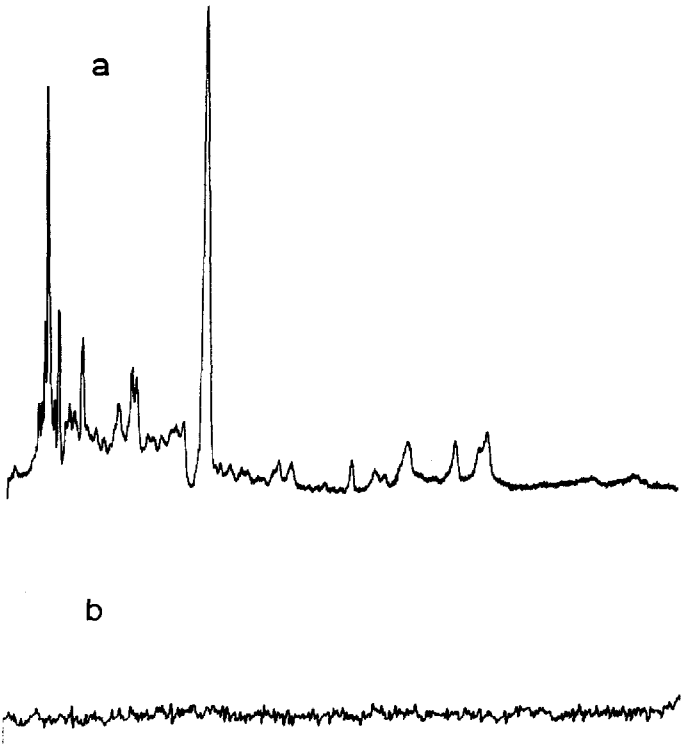

Fig. 5. Chromatograms of a hexane extract of cigarette filter. (a) Absorbance; (b) fluorescence. GC temperature program: 60°C, 2 min; 10°C/min; 280°C, 60 min.

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

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

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

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