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HYDROCARBONS FROM CARBON BLACK.

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GRADUATE COLLEGE

DIRECT IDENTIFICATION OF POLYCYCLIC AROMATIC

HYDROCARBONS FROM CARBON BLACK

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DIRECT IDENTIFICATION OF POLYCYCLIC AROMATIC
HYDROCARBONS FROM CARBON BLACK

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DIRECT IDENTIFICATION OF POLYCYCLIC AROMATIC
HYDROCARBONS FROM CARBON BLACK

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Carcinogenicity, Structure and Nomenclature

Since Percival Pott observed the scrotal cancer of chimney sweeps in 1775, there has been a continual search for the causes, prevention, and treatment of cancer (1). As early as 1850, soot and/or chimney deposits were believed to contribute in some way to this occupational disease (2).

The first chemical carcinogenesis was reported in 1918 by the Japanese workers, Yamagiwa and Ichikawa (3). Coal tar was painted on the ears of 137 rabbits every two or three days and after 360 days, there were seven cases of fully developed carcinomas. According to Shimkin and Triolo (4), Tsutsui, a student of Yamagiwa, further showed that mice and rats were susceptible to coal tar. Also Passey (5) produced exogenous skin tumors on mice from applications of the basic-ether soluble fraction of soot in 1921.

In 1932 Kennaway and co-workers (6) showed that 1,2,5,6-dibenzanthracene was the first pure substance known to produce cancer. Shimkin

and Triolo (4) compared the work of the isolation and identification of polycyclic hydrocarbons from coal tar by Kennaway, Hieger, Cook, Mayneord and Haslewood to that of the Curie's isolation of radium from pitchblende. They state:

The parallel of radium and of benzpyrene, in fact, extends even further: radioactivity was the property that facilitated the isolation of radium; the fluorescence spectrum was 'the single thread that led through this labyrinth' of coal tar.

Despite the full accounts of these events by Kennaway, mention must be made of some facts before and after the apical establishment in 1930 of carcinogenicity of polycyclic hydrocarbons. Swiss chemists demonstrated by 1926 that the active material in coal tar was concentrated in the higher boiling fractions as a neutral cyclic compound free of nitrogen, arsenic or sulfur. The observations of Mayneord, in 1927, of the fluorescent spectra of carcinogenic fractions allowed Hieger to relate these to a series of anthracene compounds. This was the clue to the selection of 1,2,5,6-dibenzanthracene as the first polycyclic hydrocarbons to be tested for carcinogenic activity, well in advance of the actual isolation and bioassay of the active tar component, 3,4-benzpyrene (4).

In 1933, Cook, Hewett and Hieger (7) isolated, synthesized, and identified the first environmental polycyclic hydrocarbon as 1,2-benzpyrene (hereafter called benzo[a]pyrene and abbreviated B[a]P). It is interesting to note that these workers started out with 2 tons of soft pitch, which had been previously shown to be carcinogenic, and isolated 24 g of impure B[a]P. Ironically, the compound recently known as 1,2-benzopyrene or benzo[e]pyrene (B[e]P) was called 4,5-benzpyrene. Even more ironically, these compounds (B[a]P and B[e]P) have been re-assigned the names originally given to them by the nomenclature rules of the International Union of Pure and Applied Chemistry (8). The numbering of pyrene by the old and new rules and the letter system (in nomenclature) is given in Figure 1 (9, 10). In the letter system, [a] is used to replace the numbers 1,2-; [b] for side 2,3-; etc. (i.e., 1,2-

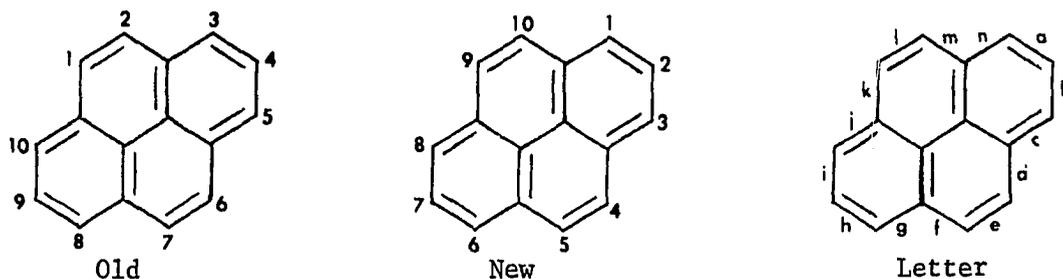


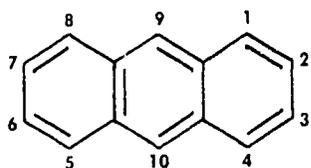
Fig. 1. Number and letter system of pyrene by International Union of Pure and Applied Chemistry.

benzopyrene or B[a]P). However, the letter system does not skip sides around the ring of a parent hydrocarbon (see perylene and benzo[ghi]-perylene in Figure 2). The numbering system, structures and names of five polycyclic aromatic hydrocarbons (Polyc Ar HCs) and related benzo-derivatives are given in Figure 2. (See Appendix A for abbreviations.)

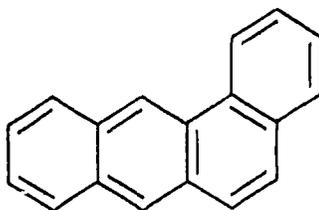
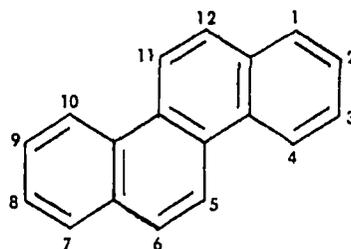
In 1939, Cottini and Mazzone (11) reported the testing of B[a]P on human subjects and concluded that if B[a]P were applied to human skin for prolonged periods, it would cause cancer. More than 1,000 animal studies testing the carcinogenicity of B[a]P have been performed through 1955 (12). Falk, Kotin and Mehler (13) also believe that B[a]P is the most ubiquitous and potent carcinogen of the Polyc Ar HCs.

Sawicki (14) lists the following air-borne Polyc Ar HCs as carcinogenic:

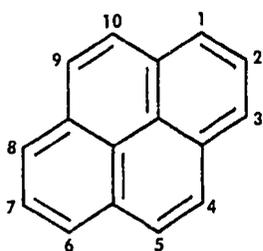
- a) benz[a]anthracene (B[a]Anth)
- b) chrysene
- c) benzo[b]fluoranthene (B[b]F)
- d) benzo[j]fluoranthene (B[j]F)
- e) B[a]P.



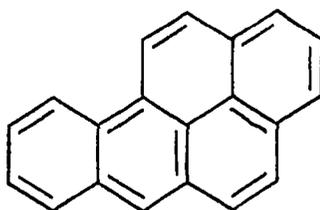
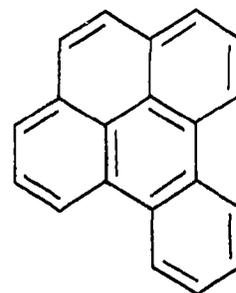
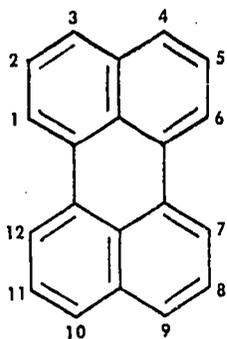
Anthracene

Benz[a]anthracene
(1,2-Benzanthracene)

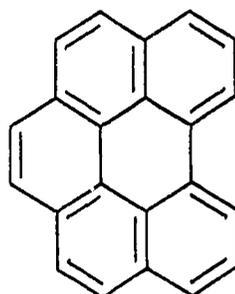
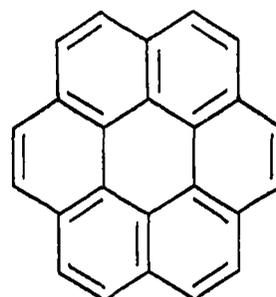
Chrysene



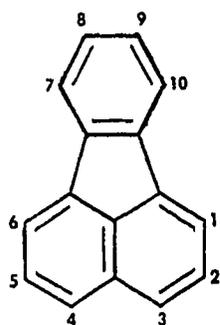
Pyrene

Benzo[a]pyrene
(1,2-Benzopyrene)Benzo[e]pyrene
(4,5-Benzopyrene)

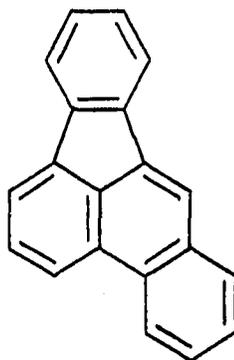
Perylene

Benzo[ghi]perylene
(1,12-Benzoperylene)

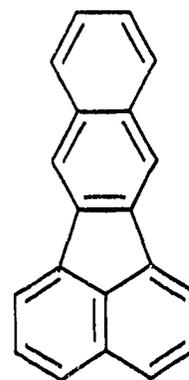
Coronene



Fluoranthene



Benzo[b]fluoranthene



Benzo[k]fluoranthene

Fig. 2. Numbering, structure, and name of five polycyclic aromatic hydrocarbons and benzo-derivatives.

Epstein, Mantel and Stanley (15) have added substituted alkyl-B[a]Anth (mostly methyl) and alkyl-chrysenes to this list.

Falk et al. (16) isolated eight Ar HCs from the benzene extracts of rubber stoppers and automobile tires and Falk and Steiner (17) identified the following seven Polyc Ar HCs from the benzene extract of furnace carbon blacks:

- a) pyrene
- b) fluoranthene (F)
- c) B[e]P
- d) B[a]P
- e) benzo[ghi]Perylene (B[ghi]Pery)
- f) anthanthrene (Anthanth)
- g) coronene

These workers list chrysene (from rubber), pyrene, and B[e]P as weak carcinogens and also B[a]P as a strong carcinogen.

Sources of Polyc Ar HCs in Our Environment

A Frenchman named Mallet (18) reported finding B[a]P at a two meter depth of marine sediment in the bay of Villefranche. The deposit was estimated to be about 500 years old by ^{14}C -dating. By further investigation he determined that B[a]P was present within the leaves of trees and plants of that area and might have been supplied to the plant from the soil where it was synthesized by anaerobic bacteria.

Two reviews on "Air Pollution" by Altshuller (19, 20) in 1967 and 1969, and Stern's book (21) give many sources of Polyc Ar HCs in air.

One of the main sources is from incomplete combustion of carbonaceous fuels--namely, coal, petroleum oil, and gas (22, 23, 24). Sawicki, Meeker and Morgan (25) reported on several Polyc Ar HC pollutants (and other polynuclear types) from residential coal furnaces, and the flue gas of an oil-gas stack of an oil refinery. Von Lehmden, Hangebrauck and Meeker (26) reported on the Polyc Ar HC emissions from several selected industrial processes. Table 1 compares the amount (wt/vol) of six Polyc Ar HC emissions from a carbon black manufacturing area, steel and coke manufacturing area, and an organic chemical industry complex to that of the residential furnaces.

TABLE 1
AMOUNTS OF POLYCYCLIC AROMATIC HYDROCARBONS IN
AIR POLLUTION SOURCE EFFLUENTS
(MICROGRAMS/1000 CU. M.)

Polyc Ar HC	Residential Coal Furnaces ^a	Carbon Black ^b (In Winter)	Steel and Coke ^b (In Summer)	Chemical (In Winter)
Pyrene	2,200,00	0.33	6.6	0.37
B[e]P	500,000	0.76	4.1	0.57
B[a]P	1,000,000	0.58	8.6	6.90
B[ghi]Pery	760,000	1.60	7.1	1.50
Anthran	190,000		0.83	
Coronene	30,000	0.59	0.44	0.78

^aStack sample

^bAir sample near source

A perspective of these pollution sources can be visualized by comparing the B[a]P concentration in Table 1 to 6 $\mu\text{g}/1000 \text{ cu m}$ of B[a]P

for the average urban air (14). With this figure in mind, one can see that residential coal furnaces contribute overwhelmingly to the pollution of air, while these other particular industrial processes are not major contributors of Polyc Ar HCs to the atmosphere.

Von Lehmden, Hangebrauck and Meeker (26) reported that certain asphalt processes (air-blowing) are an important source of pyrene and substituted alkyl polynuclear HCs, however. Cleary and Sullivan (27) of Australia believe that the Polyc Ar HC pollution due to gasoline and diesel engine exhausts will become more important in the next few years, while the contribution from coal combustion processes will decline progressively.

Conlee et al. (28) reported on the motor vehicle exhaust at three selected sites: the Sumner Tunnel, the International Peace Bridge and Urban Cincinnati. The Sumner Tunnel in Boston was considered as a large, dynamic test chamber for the estimation of air pollutants. The mean concentration (in $\mu\text{g}/1000 \text{ cu m}$) of several Polyc Ar HCs at the center of the tunnel and at the outlet air site was found to be from five to twenty times greater than at the inlet air site. The mean concentration of these same Polyc Ar HCs ranged from two to four times as much (except pyrene, which was about the same) and from three to seven times as much (except coronene, not reported) in Urban Cincinnati and at the International Peace Bridge, respectively, compared to inlet air of Sumner Tunnel. These comparisons are probably not absolutely valid, since the samples were collected at a different time of the year in 1963. However, the authors concluded that the auto exhaust appears to be a prime contributor to benzene-soluble particulates

(74) per cent), of which a high percentage are polynuclear HCs (besides several other air pollutants).

In a series of papers entitled "The Formation of Aromatic Hydrocarbons at High Temperatures, Part I-XIV," Badger and co-workers (29, 30, 31) postulated and proved a free radical mechanism for the formation of a number of the Polyc Ar HCs from lower molecular weight (mol wt) HCs in the combustion process. A summary of this work was presented at a Symposium on The Analysis of Carcinogenic Air Pollutants by Badger (32) in 1961. They pyrolyzed (separately) acetylene, butadiene, vinylcyclohexene, styrene, ethylbenzene, butylbenzene, phenylbutadiene, tetralin, benzene, toluene, propylbenzene and indene at 700° C. Each resulting tar was analyzed "by distillation, gas-liquid chromatography, chromatography on alumina and on acetylated cellulose, and by infrared, ultraviolet, and fluorescence spectroscopy". A description of their pyrolysis of acetylene follows: purified acetylene, at the rate of 6 liters per hour, was passed through a 30 in x 1.25 in, OD silica tube with the last 6 in heated to 700° C. Four hundred liters of acetylene gave 44.3 g of tar, which had the following percentage composition (wt/wt in parentheses) (30):

Benzene (28), toluene (3), o-xylene (trace), styrene (trace), indene (trace), naphthalene (12), biphenyl (2.7), fluorene (4.5), phenanthrene (13), fluoranthene (3.7), chrysene (0.5), 1,2-benzanthracene (0.4), pyrene (6.5), perylene (trace), 1,12-benzoperylene (trace), 3,4-benzopyrene (2), 2,3-o-phenylenepyrene (2.7); and intractable 'polymeric' material (20).

A similar tar was formed on heating acetylene at 530° C and on analysis, several of the preceding compounds were not detected, but indene (28), naphthalene (20.2) and pyrene (21) were found in a higher

concentration (percentage in parentheses) and B[a]P was again formed at 2 per cent. In a spectacular radioactive ^{14}C -study on the pyrolysis of [α - ^{14}C]ethylbenzene, Badger and Kimber (31) showed that the formation of naphthalene, phenanthrene, chrysene and B[b]F was consistent with their proposed free radical mechanism. Anthracene was believed to be formed by thermal rearrangement of phenanthrene and pyrene by more than one route.

The carcinogenic Polyc Ar HCs of rubber tires and furnace carbon blacks has been previously pointed out (16,17). Reports by Falk, Kotin and Miller (33) and more recently by Nau and co-workers (34, 35) gave evidence that rubber tire dust contributes to the Polyc Ar HC pollutants of our atmosphere. Thompson (36) has identified vehicle tire rubber in roadway dusts and Smith (2) has separated and identified several Polyc Ar HCs in rubber dust. Cleary and Sullivan (27) also believed that tire rubber and dust from bitumen road surfaces are significant sources of Polyc Ar HCs as air pollutants. In addition to the wear and tear of tires on the millions of cars on our highways, the disposal of rubber tires in the United States is a nationwide problem (37).

The Pcth Report (38) and the article by Lozano, Melvin and Hochheiser (39) gave evidence that the exhaust from jet airplanes contributes to the air pollution in total HCs, aromatics and particulates. In most cases, these reports show a higher total HC and Ar level at a lower power setting, but a greater amount of particulates at higher power settings. By comparing the amount of B[a]P in the exhaust gases of these jet engines with that of the average urban air, and with the

number of jet airplanes flying today, this writer believes that this is another significant source of Polyc Ar HCs in the atmosphere, even though jet aircraft was not considered a significant source of pollution in a recent report (40). A similar observation to that shown in All Clear (41), but even more dramatic, was seen at Will Rogers World Air Port, Oklahoma City, in May, 1970: as a jet plane was taking off going south on the runway, one could not see the plane before it left the ground because of the heavy cloud of black smoke it left behind.

Further, in an interesting epidemiological study in six European cities and two localities of Wales, Stocks (42) reported a high correlation of cigarette smoking plus air pollution to lung cancer mortality. The mean annual values of four Polyc Ar HCs in seven areas (he reported) do not look significantly high compared to residential coal furnaces in Table 1. However, there seemed to be a significantly higher lung cancer rate in four areas (Belfast, Dublin, Liverpool and North Wales) where the average smoke was higher (174 mg/1000 cu m) compared to areas of lower average smoke (56 mg/1000 cu m). Stocks gave a new hypothesis of the causation of lung cancer in this report: ". . . that only those who have first developed a susceptibility for cancer of the lung are affected by such factors as cigarette smoking and air pollution."

The results of another study reported by Lazar et al. (43) in which B[a]P of cigarette smoke condensate was applied to mouse skin in both short-term and long-term experiments, seem to contradict Stocks' work and work by others (11, 13). They concluded that B[a]P was not an active carcinogen of cigarette smoke condensate. Possibly this anomaly

can be partly explained by the earlier research being both on the skin and pulmonary system of the human (11, 13, 42), while the latter was only on the skin of mice.

Lyons (44) compared the Polyc Ar HCs from gasoline and diesel engine exhausts, general atmospheric dust and cigarette smoke condensate. He identified the following Polyc Ar HCs (besides two of lower mol wt) from all four sources: anthracene, pyrene, F, B[a]Anth, B[e]P, perylene, B[a]P, B[ghi]Pery, B[b]F, B[k]F, Anthan, and coronene. Besides the carcinogens listed previously, dibenzo[a,h]pyrene, dibenzo[a,e]pyrene, and dibenzo[a,h]anthracene (first cancer forming compound) were found in the exhaust of the gasoline engine. While these different sources were qualitatively similar in regard to the Polyc Ar HCs, a wide quantitative difference was found.

Sawicki (45) reported that the ratio of B[a]P : B[ghi]Pery (or B[a]P : coronene) could be used to distinguish the source of the Polyc Ar HC pollutant. The ratio is usually $< 0.6:1$ for particulate samples believed to be from automobile exhaust, whereas the ratio is usually $> 1:1$ for coal combustion pollution. Pollution from coal tar pitch fumes is usually indicated by high ratios of pyrene : B[a]P and B[a]P : B[ghi]Pery ($\geq 10:1$).

From this survey, the chief sources of atmospheric Polyc Ar HC pollutants are:

- a) Combustion of coal and other carbonaceous fuels in residential and industrial furnaces
- b) Motor vehicle exhaust from gasoline and diesel engines
- c) Certain chemical producing operations (tarring and

asphalt processes)

- d) Dust from rubber tire and asphalt disintegration
- e) Garbage incinerators and outdoor burning of car bodies, seats, etc. (14)
- f) Emissions from jet engines (planes)
- g) Smoke and tars from cigarettes (personal pollution) and smoke from outdoor burning of grass, leaves (forest fires), etc.

Extraction, Separation and Identification of Polyc Ar HCs

A general flow chart indicating the extraction, separation and identification procedure used by many workers in the Polyc Ar HC field is given in Figure 3. The numbered steps in the flow chart may be referred to in following the description. Several of the workers have omitted one or two steps or reversed their order in analyzing different types of samples (i.e., the omission of Step 2 - column chromatography - in effects of various solvents and conditions on the recovery of B[a]P by Stanley, Meeker and Morgan (46)).

Step 1. Extraction of the organic soluble material from a weighed sample of carbon black or other sample (containing Polyc Ar HCs) has been carried out with a suitable solvent (benzene, cyclohexane, acetone, or methylene chloride, etc.) in a Soxhlet extractor for a set period of time, usually 4 to 24 hrs (16, 25, 26, 27, 35, 46, 47, 48). Part of the solvent containing the extracted Polyc Ar HCs was evaporated or distilled and then diluted to a known volume. The separation and identification of Polyc Ar HCs were performed on a sample from this prepared solution.

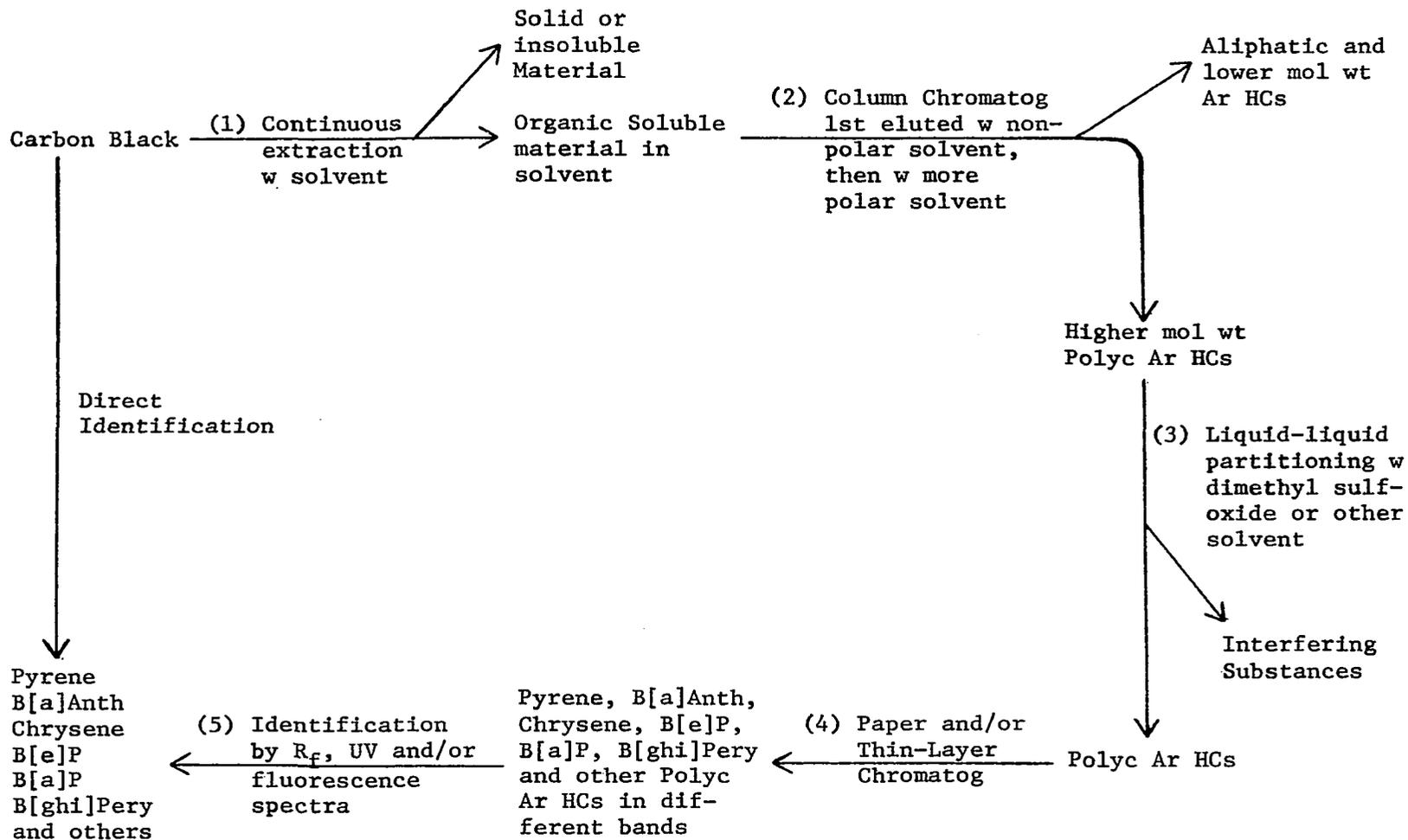


Fig. 3. Flow chart for extraction, separation and identification of Polycyclic Aromatic Hydrocarbons.

Step 2. A gross separation of the aliphatic and lower mol wt Ar HCs from the higher mol wt Ar HCs has been successfully performed by liquid chromatography (chromatog) in short columns with a suitable adsorbent (33, 47, 48, 49, 50, 51, 52, 53, 54, 55). Longer columns for complete separation of the desired Polyc Ar HCs have been used by some workers with limited success (16, 17, 27, 56, 57).

Step 3. Liquid-liquid partitioning (extraction in separatory funnels) with dimethyl sulfoxide has been carried out by Howard and co-workers (52, 53, 54, 55) in the estimation of Polyc Ar HCs in smoked foods, vegetable oils and total diet composites. Smith, Nau and Lawrence (35) used this technique with N,N-dimethylformamide to remove interfering substances in the identification of Polyc Ar HCs in rubber dust. A similar extraction process was also used in the analysis of B[a]P in cigarette smoke (49).

Step 4. Paper chromatog has been used for further separation of the Polyc Ar HCs (27, 48, 49, 52, 54). But more recently, thin-layer chromatog seems to be replacing paper chromatog, possibly because the desired separation was not achieved, and paper chromatog is very time consuming (4-8 hrs). Thin-layer chromatog has been used principally for the isolation of B[a]P from B[e]P, B[k]F, and perylene by many investigators (35, 46, 52, 53, 54, 55, 58). White and Howard (59) have determined the R_f values for 29 Polyc Ar HCs on cellulose and cellulose acetate thin-layer plates. R_f has the same meaning in both paper and thin-layer chromatog and ". . . is defined as the ratio of the distance traveled by the compound to the distance traveled by the solvent" (60). Sawicki et al. (61) have examined and identified B[a]P,

B[e]P, and B[k]F also by R_f values in two different solvent systems on thin-layer plates along with the fluorometric spectra (compared to standard spectra).

Step 5. Identifications have been made by comparing the R_f value on a thin-layer plate (or paper chromatogram) and the UV spectrum of the suspected Polyc Ar HC with those (R_f and UV spectrum) of a standard (Std) sample of the authentic HC, respectively (35, 50, 51, 59). UV spectra have been used for the identification of the carcinogen, B[a]P, by many workers in this field for about 35 years (16, 17, 26, 27, 33, 46, 50, 51, 52, 53, 54, 55, 58, 59).

More recently (15 yrs), spectrophotofluorometry (along with UV spectra in some cases) has been used for the identification of Polyc Ar HCs (33, 39, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 58, 61). In most cases, the fluorometric method is more specific and much more sensitive than the UV method of analysis. A few of the more striking documented cases are cited. Thomas et al. (62) presented a paper at the Air Pollution Symposium in 1959 on the fluorescent spectra of Polyc Ar HCs found in polluted atmosphere. They stated: "Fluorescent spectrometry offers a very sensitive method of both qualitative and quantitative analysis as, in comparison with other types of spectral analysis, only very small quantities of material are required," and this is substantiated by Zdrojewski et al.'s (63) remark, "Rated conservatively, there is a sensitivity differential of more than 100 times in favor of fluorescence measurements" (they were comparing UV vs. fluorescence spectra in analysis of B[a]P and B[k]F). Sawicki, Hauser and Stanley (64) claimed that B[a]P can be unequivocally identified in

a mixture of 50 Ar and polynuclear HCs by the destructive fluorescent method in sulfuric acid and Howard et al. (53) claimed positive identification of B[a]P in food as low as 0.02 ppb from its fluorescence spectrum! Jager (65) has reported the spectral measurements of the benzpyrene fraction (B[a]P, B[ghi]Pery, Anthran, perylene and B[k]F) from air-borne particulates in n-heptane at the boiling point of liquid nitrogen. He called this a quasilinear method and believed ". . . it will become not only one of the most sensitive but also one of the most selective methods of the analysis of polycyclic aromatic hydrocarbons."

Sawicki et al. (66) compared eleven methods for the analysis of B[a]P (and several other Polyc Ar HCs) which considered the five steps previously mentioned, but not simultaneously. They compared precision, accuracy, man-hrs of work and total analysis time. They also made recommendations for which method should be used under a given circumstance. Most of these methods for analysis of the Polyc Ar HCs were time consuming (1-4 days) and laborious.

Monkman, Moore and Katz (67) believed that the Soxhlet extraction and column chromatog could be eliminated, since the sample is exposed to heat, light and air which may destroy or oxidize some of the Polyc Ar HC content. They extracted the air particulate pollutants from a glass fiber sheet for 3 hrs at room temperature with cyclohexane and went directly to the spectrophotofluorometer (SPF) for identification and quantitation. The Soxhlet extraction method was also avoided by a vacuum sublimation method of the Polyc Ar HCs from air particulates by the Japanese workers, Arito, Soda, and Matsushita (68). They

reported a recovery of 102 ± 7 , 90 ± 4 and 97 ± 3 per cent for pyrene, B[a]P and perylene, respectively.

Sawicki et al. (66) further suggested improvement on the separability and analysis of the family of Polyc Ar HCs by gas chromatography (GC). The gas chromatographic method does have the advantage of speed over most of the previously mentioned methods. It therefore appears that progress could be made by some direct identification method (indicated by direct arrow in Figure 3) for the Polyc Ar HCs.

Earlier work in 1961 by Dupire (69) showed the potentiality of GC in the Polyc Ar HC area by obtaining satisfactory results on coal tar products (raw tar, distilled oils, and road tars). He used a high temperature column (up to 360° C) and identified most of the collected effluents (peaks) by infrared analysis in solvent (isooctane) or in potassium bromide discs. Several Italian workers showed that some of the Polyc Ar HCs in dust samples could be analyzed by judicious selection of the liquid phase (stationary) on the solid support (70, 71). In the latter paper, they compared the response of the electron capture detector with that of flame ionization (FI) detector and experimented with capillary columns. They showed that temperature programming was advantageous for separation of compounds with relatively short retention times and prevented lagging of those with longer times. Arito, Soda and Matsushita (68) also used GC for analysis of seven HCs from air particulates. DeMaio and Corn (72, 73) used dual columns for the first time to analyze air-borne particulates for Polyc Ar HC content. The dual columns were used to allow for the bleeding of the liquid phase at the high temperatures used. Wilmshurst (74) gave a

list of previous references that were not suited to low-level analysis as is encountered in air pollution studies. He used the FI detector and did preliminary work with the argon detector and further investigated the use of capillary columns. Carugno and Rossi (75) found that a capillary column was capable of separating a large number of polynuclear HCs from a complex mixture. They applied the method to cigarette smoke condensate, used both isothermal and temperature programming for the column and used both FI and electron capture detectors. The electron capture detector was found to give a better response for some Polyc Ar HCs, namely B[a]P and B[e]P (71, 75). Other workers who have used the electron capture detector in GC studies of cigarette smoke are Governator et al. (76) and Davis (77). Because of the low temperature (225° C) of the highest setting for electron capture detectors with a tritium source, Davis (77) used a helium glow discharge as the electron source that could be operated up to 400° C. He claimed that 1 nanogram (ng) of B[a]P could be measured and showed that the analyses by GC were in good agreement with those of a fluorometric method. Governator et al. (76) claimed detection of as little as 10 ng of B[a]P and similar amounts for other polycyclics. They also evaluated several liquid phases and solid supports capable of separating B[a]P from B[e]P and found a 5 per cent Silicone Rubber, SE-30 on 30/40 mesh Anakrom ABS in a 15 ft x 0.125 in OD (the outside diameter of column unless stated otherwise), stainless steel (SS) column to be the best for their conditions.

Chakraborty and Long (78) burned ethylene and ethane (separately) in a diffusion flame to produce soots, which were then analyzed

for Polycyclic Aromatic Hydrocarbons (PAHs) by GC. They developed a gas chromatogram using dual, 12 ft x 0.25 in, SE-52 columns and their identification of the compound(s) in the collected peaks closely parallels the initial results of this study. These results will be discussed further in the chapter on "Results and Discussion" of this dissertation. They collected the peaks by bubbling the effluent helium (He) carrier gas through hexane in a test tube after it had passed through the exit port of the thermal conductivity detector. For quantitative analysis and better separations, they used dual, 50 ft x 0.125 in, SE-52 Hi-Pak columns. However, with these columns the components of every peak were still not resolved.

One of the more recent, sophisticated applications of GC was reported by Bowman and Beroza (79). They combined the high separative powers of GC with the high sensitivity and selectivity of the SPF and used a flow cell with ethanol as solvent. The apparatus was used to measure pesticides, air pollutants and methylenedioxyphenyl compounds. The detection limits (in ng) for the following PAHs were reported: fluorene - 0.9, anthracene - 10, p-terphenyl - 0.4, chrysene - 9, and B[a]P - 0.7.

Duncan (80) has recently reported the use of a mixture of sodium chloride with the liquid phase SE-30, on Chromosorb G to separate B[a]P from both B[e]P and B[k]F with an electron capture detector. The analysis time was short and he reported more reliable results than were obtained by existing methods. He also gave a list of 18 different columns, with five different liquid phases on a variety of solid supports, that were tried for the separation of B[a]P from B[e]P.

The silicone gum rubbers of SE-30 and SE-52 have been used as the liquid phase for GC work in the Polyc Ar HC area as reported by most researchers. The specifications of most of the columns used (as found in this survey) for Polyc Ar HC separations are summarized in Table 2. It was of interest to note that four of these groups of workers used nitrogen (N_2) and two used He as carrier gas and two authors did not report the gas used.

The analysis of nonvolatile materials has been accomplished with GC and an attached pyrolyzer since about 1960. Barbour (81), and Honaker and Horton (82) have pyrolyzed polymers and analyzed the pyrolyzate by GC. Thompson (36) has used this method in the identification of styrene from styrene-butadiene rubber in roadway dusts and the method has been used to characterize the organic constituents of brake linings (83). According to Perry (84), Hewitt and Whitham summarized several advantages in using GC for examination of pyrolysis products. These listed advantages were:

- a) "The products of pyrolysis are rapidly separated."
- b) "The separation can easily be recorded automatically."
- c) "Only a few milligrams of sample are required."
- d) "Products of pyrolysis can be identified by their retention time."
- e) "Further identification of the products of pyrolysis can be obtained by collecting fractions at the outlet from the column."
- f) "The chromatogram of the products of pyrolysis is a fingerprint."
- g) "The pyrolysis unit may be connected directly to the inlet of the chromatographic column."

A review by Perry (85) listed other materials (e.g., bacteria, some drugs, etc.) that have been examined by pyrolysis-GC, discussed pyrolysis techniques and Curie-point pyrolyzers, discussed sample

TABLE 2
SPECIFICATIONS OF COLUMNS AND REFERENCE NUMBERS IN THIS LITERATURE REVIEW

Length	Diameter (OD, unless otherwise given)	Tubing Material	Percentage Loading of Liquid Phase	Liquid Phase	Mesh Size	Solid Support	Reference No.
12 ft	0.125 in	SS	1	SE-30	60-80	Diasolid H	68
6 ft (dual)	0.125 in	SS	2	Apiezon L	60-80	Diatoport S	73
20 ft (dual)	0.125	Copper	2	SE-30	60-80	Gas Chrom Z	
2 & 10 m	2 & 4 mm (ID)	SS	10	SE-30 & SE-52	60-80	Chromosorb W	74
60 m	0.5 mm (ID)	SS Cap	5	SE-30			
15 ft	0.125 in	SS	5	SE-30	30-40	Anakrom ABS	76
9 ft	0.125 in	SS	3	SE-30	60-80	Chromosorb W	77
12 ft (dual)	0.25 in	SS ^a	10	SE-52	60-80	Chromosorb W	78
50 ft (dual)	0.125 in	SS	b	SE-52	100	Chromosorb G	
1.25 m	4 mm (ID)	Glass	5	QF-1	80-100	Gas Chrom Q	79
10 ft	0.125 in	SS	2	SE-30	48-65 60-80	NaCl (40%) & Chromosorb G	80

^aNot reported, but believed to be SS

^bNot reported in this article

requirements and standardization of fingerprint chromatograms (called pyrograms), and gave many applications, including some in our area of environmental health. He believed the pyrolysis-GC is ubiquitous today because of its easy and successful application in the identification of a wide variety of nonvolatile materials by fingerprinting. In this review he further states:

The biochemical applications of PGC as of many analytical techniques look likely to make enormous contributions to the health and well-being of homo sapiens and to increase his knowledge of the universe. The most sensational practical advances are likely to come in these fields.

All one has to do is to glance at the very recent GC review to realize it is a lively field (86). In this review, the American Chemical Society has reported (from 1968 National Register of Scientific and Technical Personnel) that ". . . 16 per cent of the analytical chemists list chromatographic analysis as their first specialty choice, a number greater than any other area of analytical chemistry." New ancillary techniques include direct linkage of GC to a mass spectrometer, to infrared and Raman spectrometer, and to a nuclear magnetic resonance spectrometer. Probably one of the most novel applications and of ultimate sophistication is the proposed experiment for the biological exploration of Mars with the pyrolysis-GC-mass spectrometer linkage.

Carbon Black

Carbon black may be defined as an amorphous form of finely divided carbon produced commercially by partial combustion or thermal decomposition of natural gas or petroleum products. About 90 per cent of the carbon black produced in the United States is used in reinforcing

rubber. Other uses are as a pigment in the ink and paint industry, coloring for certain foods and in dark cosmetics.

The classification of the carbon blacks is based on their method of preparation. Smith (87) gave the four types as:

- a) Channel blacks (or impingement blacks) are prepared by the impingement of small natural gas flames.
- b) Furnace combustion blacks are produced by the partial combustion of mostly gaseous HCs in a closed retort or furnace.
- c) Furnace thermal blacks are produced by thermal decomposition of HCs in preheated furnaces.
- d) Lampblacks are prepared by burning liquid fuels (petroleum oils, tars and Ar residues) in specially designed pans.

The principal uses of carbon blacks depend on their physical and chemical properties; namely, particle size, anisotropy and surface characteristics. Electron microscopic studies showed that the particles are essentially spherical. The channel blacks have the smaller particle size which may vary from 50 to 350 A° in diameter, the furnace combustion blacks range from 500 to 800 A° and the furnace thermal blacks from 1,000 to 3,000 A° in diameter. The channel blacks used for reinforcing rubber are about 250 to 350 A° in diameter and the furnace combustion blacks (of size above) are used for semireinforcing rubber.

Carbon black is essentially carbon combined with residual hydrogen and chemically combined oxygen on its surface.

The amount of oxygen present has an effect on the properties of the black--the more chemisorbed oxygen the greater the hydrophilic property of the black and the more acidic the water sludge of this black becomes. The properties imparted by the chemisorbed oxygen are very important to the rubber industry. The chemisorbed oxygen and hydrogen present on carbon black are termed "volatile matter" and may be determined by heating most blacks to 1200 C (88).

Other chemical properties of carbon blacks are the pH, ash content, benzene extractable material, and iodine adsorption. The ash content consists primarily of the oxides of iron, silicon and magnesium and is believed to arise from the hard water used for quenching. The ash content reported for channel black was usually < 0.1 and for furnace black about 0.1 - 1.0 per cent (87). The channel carbon blacks have very little benzene extractable material, while about 0.1 - 0.3 per cent of the furnace blacks are extractable with hot benzene (88, 89). The surface area (in sq m/g) may be determined from the iodine adsorption and the particle size approximated from this surface area (87).

The nomenclature and properties and the many and varied uses of carbon black have been summarized by Nau and co-workers (88, 90). Sweitzer (91) has reported (in tables) the uses of carbon blacks based on their primary properties of (a) fineness, (b) adsorption, (c) absorption, (d) blackness, (e) radiation absorption, (f) electrical conductivity, and (g) chemical reactivity.

Before 1940 channel blacks accounted for most of the domestic carbon black produced, but the channel process now (last ten years) accounts for less than 20 per cent. The price increase of natural gas along with the superior performance of oil furnace blacks in tire manufacture have contributed to this shift. The rubber industry (mainly

tires) and carbon black production in the United States are very closely related. The weight percentage of carbon black in rubber used for tires is about 30 to 50 (87). It not only reinforces the rubber tire, but also increases the length of the tread wear about ten times. Styrene-butadiene rubber is still the major elastomer used in rubber tires, and as dramatically pointed out, "This 27-year-old, World War II baby may have slowed down, but there still is life left in it" (92).

The producers have been able to keep up with the rising price by increasing the master-batching oil from 8 to 18.6 per cent of the total styrene-butadiene rubber production since 1955. Anderson (92) further predicts an increase in master-batching oil to about 20 per cent by 1974 and states:

The pace will depend upon how fast carbon black producers will be able to supply finer structured blacks and how rapidly processing techniques can be developed to intimately mix the master-batch. The rule is, the higher the carbon black content, the more it can be extended with oil.

Carbon black production in the United States has increased from 1.8 billion lbs in 1957 to almost 3 billion in 1969 (93). About 2.6 billion lbs were used by the rubber industries at a value of more than \$210 million. He predicts an increase in carbon black consumption by the rubber industry of about 3 per cent (2.7 billion lbs) and nonrubber uses (mainly in inks and paints) of about 180 million lbs in 1970.

The possibility of a new market for rubber products (requiring more carbon black) in the near future is rubber roads (92). The addition of small amounts of rubber to an asphalt paving material (already carbonaceous material) is believed to lengthen the life of a

road several years before maintenance is required. Research by Good-year has shown that styrene-butadiene rubber, with high mol wt and low gel content, is the best polymer for rubber-asphalt mixes.

Carbon black and benzene extracts of it (channel and furnace) have been tested for carcinogenicity by ingestion, skin contact and subcutaneous injections on mice (some on rabbits, guinea pigs and monkeys) by Nau and co-workers (88, 90, 94). The benzene extract of some furnace blacks produced tumors in the stomach, on the skin, and in the tissue of mice in these tests. These authors believed that the benzene extract contained a substance (or substances), which when removed from the carbon black, produced cancer in mice. Nau et al. (89) later expanded this work to include inhalation studies of carbon black on mice, hamsters, guinea pigs, rabbits and monkeys. The first report of these experiments showed no malignancies, even though gross and microscopic changes in the lungs of mice and monkeys were noted.

At least three groups of researchers: Falk and co-workers (17, 33), Nau and co-workers (90, 95) and Tomingas and co-workers (96), have done elution and/or adsorption studies of the Polyc Ar HCs with carbon black. There has been some controversy about the carcinogenicity of the soots and/or carbon black particulates in the atmosphere on the skin and pulmonary system of humans and also some disagreement about the elution of the polycyclics from carbon black.

Based on clinical, epidemiological and statistical evidence up to 1952, Falk and Steiner (17) believed that soot polluted air did not unequivocally present a hazard. Falk, Kotin and Miller (33) state, "The liberation of benzpyrene from rubber tire dust by an

aqueous protein solution, as encountered in the lung, occurs rapidly."

Conversely, the Polyc Ar HCs were not eluted to a significant amount from a number of channel and furnace carbon blacks by (a) human blood plasma, (b) artificial gastric juice and (c) artificial intestinal juice, as found by Nau and co-workers (85). Neal, Thorton and Nau (95) further did not find any significant elution of the HCs from a test rubber sheet by a number of food solvents or products, namely: (a) cottonseed oil, (b) aqueous citric acid, pH of 3.85, (c) whole, homogenized milk and (d) a 3 per cent aqueous solution of each acetic acid, sodium bicarbonate and sodium chloride. These authors showed that either channel or furnace carbon blacks can adsorb (in certain cases-- on ingestion, on skin contact and on subcutaneous injections) and hold the carcinogenic Polyc Ar HCs so strongly that no tumors were formed.

Falk and Steiner (97) reported that there was some correlation between the surface area and the adsorptive powers of the active carbon black and claimed that the tenth extraction of a furnace carbon black yielded nearly as much Polyc Ar HC as the first. Further work on this dilemma by Kutscher, Tomingas and Petkauskas (96) indicated that the adsorption and retention of soot (and two types of carbon) were affected by their surface characteristics, but were independent of the particle size (which partly disagrees with the work of Falk and Steiner (97)). These German workers claimed that ". . . continuous extraction can remove all of B[a]P from soot, and this indicates that all the carcinogenic materials will be extracted from soot during the long period (of order of 25 years) available for this in the human body" (96).

This could possibly explain the long incubation period of lung cancer due to cigarette smoking and/or air pollutants.

Since Passey (5) showed that an ether soluble fraction of soot produced cancer on mice in 1921, there have been several other reports (in addition to the ones given above) of carbon black or soot extracts producing tumors in laboratory animals. Falk and co-workers (16, 17, 33) and Smith, Nau and Lawrence (35) have identified several Polycyclic Aromatic Hydrocarbons (PAHs) from carbon black or rubber (carbon black derived substance) by long and tedious methods and some of these identified compounds have been shown to be carcinogenic.

CHAPTER II

PURPOSE AND SCOPE

There is a good prospect that the American public will continue using the many and varied rubber products in their every day life. Two facts now are fairly certain: (a) the carcinogenic Polycyclic Aromatic Hydrocarbons (PAHs) of the environment are a public health hazard, and (b) the soot or carbonaceous particulates of the atmosphere harbor Polycyclic Aromatic Hydrocarbons (PAHs). The absolute origin of the particulates still remains to be shown, but two of the major sources are incomplete combustion of carbonaceous fuels and the degradative products associated with motor vehicles. The latter includes rubber products (mainly tires), fuels and oils, and the paved asphalt (or tar) highways.

The Environmental Health Department (formerly Institute of Environmental Health) at the University of Oklahoma Medical Center has been conducting animal studies with carbon black and rubber dust since 1962. Carbon black and rubber dust have been shown to contain at least four Polycyclic Aromatic Hydrocarbons (PAHs) that are carcinogenic in laboratory animals; and in addition Benzo[a]pyrene (BaP) is believed to be a carcinogen in humans. Therefore, the Polycyclic Aromatic Hydrocarbons (PAHs) in carbon black and rubber dust must be analyzed and a more direct method of identification seems desirable.

In the past, the identification of Polycyclic Aromatic Hydrocarbons (PAHs) in carbon black,

rubber products and polluted air samples have involved one or more extraction procedures and one or more methods of chromatographic separation. The analyses were long and tedious and in many cases only a very small amount of relatively pure compound(s) was obtained for spectral comparison with the authentic HC. Even though much progress has been made in the last ten years, Sawicki (14) suggested that improved methods for the trace analysis (from ng to μ g concentrations) of the Polycyclic Aromatic Hydrocarbons (PAHs) be made on μ g to mg amounts of particulate. Sawicki *et al.* (66) further suggested the use of GC as a method for improving the separation and analysis of this group of organic HCs. In most cases, GC requires more elaborate sample preparation and more frequent maintenance time, but after the columns have been selected and the optimum operating conditions have been found, it is faster than most existing previous methods (98).

The gas chromatogram of a carbon black sample by Thompson (34) with an attached pyrolyzer and the sublimation of the Polycyclic Aromatic Hydrocarbons (PAHs) from the dust particulates by Arito, Soda and Matsushita (68), suggested the exploration of these techniques in combination with GC for a shorter method of analysis. Therefore the main objective of this study was to develop a faster, more direct method of identification for the Polycyclic Aromatic Hydrocarbons (PAHs) from carbon black.

CHAPTER III

MATERIALS, INSTRUMENTS AND PROCEDURES

Carbon Black

The carbon black used in this study was a fine furnace thermal black with which the Environmental Health Department is conducting research. The particle size of this type is in the range of 1200-2000 Å in diameter and the surface area is in the order of 15 sq m/g.

A weighed sample of this carbon black was extracted with benzene in a Soxhlet apparatus, concentrated and used for injection into the GC for initial development of chromatograms. Later, chromatograms were developed with a carbon disulfide (CS₂) solution of the benzene extract of carbon black. The benzene of the extract was displaced with isooctane (i-C₈) (2 rinsings of 4-5 ml) on the steam bath with a N₂ jet and then evaporated to dryness with a jet of N₂ while being kept slightly warm. The residue was dissolved in about 2 ml of CS₂, the CS₂ evaporated to dryness in a warm water bath with a N₂ jet and the reddish brown residue dissolved in 2 ml of CS₂.

Other small samples (1.5 - 3.0 mg) of carbon black were placed into capillary tubes (with one end sealed), and each placed in the probe of a pyrolyzer unit to develop chromatograms by direct desorption of the HCs in the gas chromatograph. The gas chromatograms by the

two methods were then compared.

Solvents, Polyc Ar HCs and Qualitative Standard Solutions

The solvents used in this study are given with their grade, source and any special purification or treatment needed. Benzene, methanol (absolute), ether (anhydrous), and CS₂ were 'Bakers Analyzed' Reagents from J. T. Baker Chemical Co., i-C₈ was 99 mole per cent from Phillips 66, and N,N-dimethylformamide was Spectro Grade from Eastman Organic Chemicals. The ether and N,N-dimethylformamide were used as received; benzene, methanol and i-C₈ were treated and distilled as given by Howard et al. (54), except the i-C₈ used for SPF studies was further washed with a potassium hydroxide solution and then with distilled, deionized water; and CS₂ was redistilled, bp 45.2-45.5° C, before use.

The following Polyc Ar HCs were used, their grade and sources are given: anthracene, 98 per cent plus, B[e]P, 97 per cent plus, mp 179-180° C, and B[ghi]Pery, 99 per cent plus were obtained from Aldrich Chemical Co., Inc; pyrene and chrysene, both Reagent - 98 plus per cent, and F, practical, from Eastman Organic Chemicals; Anthan, and coronene, both 95-99 per cent from K & K Laboratories, Inc.; B[a]P, Baker Grade (high purity), mp 176-178° C, from J. T. Baker Chemical Co.; and B[a]Anth, high purity from Nutritional Biochemicals Corporation.

Qualitative Std solutions of the Polyc Ar HCs in CS₂ and i-C₈ were prepared for GC and SPF work, respectively. The Stds in CS₂ were prepared by transferring the desired volume (with pipet) of previously prepared Stds in benzene into 50 ml ground-glass stoppered Erlenmeyer

flasks (unless otherwise given, ground-glass stoppers and ground-glass connections, for distillations and extractions, were used throughout). The benzene solution was evaporated to about 1 ml while on the steam bath with a N_2 jet and the remaining benzene was displaced by rinsing the flask down with $i-C_8$ at least four times with evaporation to a small volume with a N_2 jet while on the steam bath. The remaining $i-C_8$ was evaporated with a N_2 jet while intermittently taking the flask off the steam bath, yet keeping it warm enough to facilitate the complete evaporation. The flask containing the residual Polyc Ar HC was rinsed down three times with CS_2 with evaporation to dryness after each CS_2 addition in a warm water bath with a N_2 jet. Then the appropriate volume of CS_2 was added to give the desired concentration, or the CS_2 solution was transferred quantitatively to a small volumetric flask and diluted to the mark with CS_2 . The following qualitative Std solutions in CS_2 were prepared: anthracene, F, pyrene, B[a]Anth, chrysene, B[a]P, B[e]P, B[ghi]Pery, Anthan and coronene. The concentration of the Polyc Ar HCs in these Stds ranged from 14 to 61 $\mu\text{g}/\text{ml}$ and they were injected into the gas chromatograph for retention time (t_R) measurements.

The Polyc Ar HC Std solutions in $i-C_8$ were prepared similarly, with the benzene being displaced first by three rinsings with regular $i-C_8$ while on the steam bath and a N_2 jet and then with two further rinsings of $i-C_8$ used for SPF work. The $i-C_8$ solutions (about 1 ml) containing the Polyc Ar HC were transferred quantitatively to small volumetric flasks and diluted to the mark with $i-C_8$. Qualitative Std solutions of the same ten Polyc Ar HCs (as prepared in CS_2) in $i-C_8$ were prepared and their concentrations ranged from 0.12 to 0.25 $\mu\text{g}/\text{ml}$

(except B[a]P - 0.008, 0.04 and 0.40 $\mu\text{g}/\text{ml}$). The excitation (Ex) and fluorescence (Fl) spectrum of each Polyc Ar HC were recorded (from 200 to 800 nanometers (nm)) with the SPF.

Instruments and Procedures

Gas Chromatograph

The F & M 810-19 Model Research Gas Chromatograph at the Environmental Health Department was used in the initial phase of the GC work. This is a dual column, dual detector instrument, which is aptly described in detail by Thompson (36). Due to instability of the baseline of the FI detector (from unknown causes) of this instrument, most of the research was done in the CAMI Building at the Federal Aviation Administration Center in southwestern Oklahoma City.

The instrument used was a Hewlett-Packard (F & M) 5750 Research Gas Chromatograph, dual column with dual thermal conductivity (TC) and FI detectors (99). The functional systems that make up the whole Research Gas Chromatograph are the programmer drawer module, electrometer drawer module and recorder, TC drawer module and oven module

Programmer drawer module. The programmer drawer module contains the electrical circuitry and controls (switches and knobs) necessary to program and regulate the temperature of the oven and thus the temperature of the columns. It also contains a temperature selector control which may be turned to read the temperature of the injection port, oven, and detectors on a pyrometer.

Electrometer drawer module and recorder. The heater controls

for the injection port, FI detector, an auxiliary heater and the electrometer are located in the electrometer drawer module. When very small currents are generated by the FI detector, a significant voltage is developed when the current flows through the input resistor of the electrometer.

The primary function of the ELECTROMETER then, is to convert the voltage signal described above into an identical voltage developed across a low resistance attenuator output which is compatible with the recorder requirements (99).

A Hewlett-Packard (Moseley) model 7128A Strip Chart dual channel recorder was used. The electrometer drawer module has a range and attenuation control (in addition to mode, zero polarity, balance, detector input and coarse and fine controls) which are used in conjunction with the recorder. The operator can select the sensitivity range at the input of the electrometer with the range control. The most sensitive setting connects a 5×10^{10} ohms input resistor across the electrometer grid and is obtained with range "1". As seen from Ohm's Law ($E = IR$), if a current of 10^{-11} amperes flowed through this resistance, a potential of 0.5 volt would be developed. Most of this chromatographic work was done with a range setting of "10", which is one-tenth as sensitive as "1", so the full scale reading on the recorder was 0.05 volt (from Ohm's Law and resistance equals to 5×10^9 ohms). The attenuation allows the operator to reduce the electrometer output voltage to the recorder and thus prevent peaks from going off scale. An attenuation scale of "1" is the most sensitive, with a setting of "2" giving a peak of one-half the height, etc.

TC drawer module. The TC drawer module contains the control

switches and the necessary circuitry to establish and monitor the detector and heated transfer zone temperatures (TC oven) ". . . as well as the necessary circuitry to balance the Wheatstone Bridge formed by the detector cell FILAMENTS" (99). The power supply for the detector filaments are in the TC drawer. The operator can set the detector and auxiliary temperatures and the bridge current from the controls on the front panel of the drawer. The amperage of the direct current supplied to the detector bridge is controlled by the bridge current setting. An increase in amperage at a given temperature will increase the sensitivity of the TC detector, but bridge current settings that are too high for a given temperature will decrease the life of the filaments. The front panel of this drawer also has a coarse and fine control, a polarity and an attenuation control. The function of the coarse, fine and attenuation controls is essentially the same as that for the FI detector, but the polarity control allows the operator to switch the polarity of the bridge output to the recorder, depending on which system ("A" or "B" injection port, column and detector) the sample was injected.

Oven module. The oven module houses the gas systems, flow controllers and rotometers, the oven and columns, the injection port assembly, the splitters and detectors. It also contains the necessary electronic components and cables connected to the various control modules.

The injection port contains a cylindrical tube, called an insert liner, into which the sample was injected at one end through a rubber septum with a syringe. The main function of the injection port

is to instantaneously vaporize the injected sample and carry it onto the column in the form of a plug by the carrier gas. DeMaio and Corn (73) give an optimum temperature of 310° C for the injection port in Polyc Ar HC analysis. The carrier gas passes through the flow control valves and the rotometers (a flow device used to monitor the flow) and then into the insert liners at the septum entrance. The insert liner used in this study was of SS and 0.25 in, OD, so the pyrolysis probe could be inserted into the "A" liner.

Column and gas-liquid chromatog separations. The column is the single, most important part in a gas chromatograph. Packed columns consist of a stationary phase on an inert solid support packed in metal (or glass) tubing of a desired length and diameter. Capillary columns are of long, fine diameter tubing (usually glass) with a thin layer of liquid phase coated on the inner surface. The most common form of GC used is still gas-liquid chromatog, so the basic principles will be explained with this type in mind (99, 100).

The stationary phase (liquid phase) is a stable, nonvolatile liquid (at least at the temperature the column is used) coated on a solid support. The solid support is a stable, inert material which usually has a large surface area, such as diatomaceous earth. "A support having a mesh range of 80 to 100 provides a good compromise between column efficiency and pressure drop," but mesh sizes from 20 to 140 have been used (99). The mobile phase is the carrier gas within the column, usually N₂ or He, which pushes the sample molecules through it.

After the sample is on the column, the molecules of the

compounds in the sample are distributed between the liquid and mobile phase as it moves through the column. Compounds will be separated only if they have different partition coefficients for a given gas-liquid system (column). The definition of the partition coefficient (K) is (101):

$$K = \frac{\text{amount of compound per volume of stationary phase}}{\text{amount of compound per volume of mobile phase.}}$$

Compounds with small partition coefficients have a larger amount dissolved in the mobile phase and therefore, would pass through the column quicker than a compound with a large coefficient. Thus separation may be achieved and different compounds will be eluted through the detector at a different time after injection (called retention time). The partition coefficient may be influenced by several factors of which some of the more important are: (a) bp of compound (vapor pressure), (b) solubility of compound in liquid phase, (c) the size and symmetry of the molecules of the compound and (d) the covalent and/or ionic character of the molecules (polarity).

The specific columns used in this research are summarized in Table 3.

The 24 ft x 0.125 in, SS columns were used as dual columns in the initial work, but were rejected because of slow carrier gas flow rates and/or leaks due to high pressure gauge settings (80 or 100 lbs/sq in).

The development of chromatograms with the 4 ft glass, SE-30 column (in "A" position) and 4 ft SS column in "B" in the oven of the F & M 810-19 Research Gas Chromatograph was attempted (used as dual

TABLE 3
SPECIFICATIONS OF COLUMNS USED

Length (ft)	Diameter OD (in)	Tubing Material	% Loaded and Liquid Phase	Mesh Size	Solid Support
24	0.125	SS	5 SE-52	100-120	Chromosorb G High Perform- ance, AW- DMCS ^a
4	0.25	SS	3.8 SE-30	80-100	Chromosorb W AW-DMCS
4	0.25 (6 mm)	Glass	3.8 SE-30	80-100	

^aAcid Washed and Dimethyldichlorosilane treated.

columns). The glass column was an "on column" injection type (injection port end of column is used directly as the insert liner), but the FI detector baseline was unstable at the high sensitivities used.

The chromatograms developed and t_R s reported in this study were made with the 4 ft x 0.25 in, SS, 3.8 per cent Silicone gum rubber, SE-30 column in the "A" position and an 18 in x 0.125 in, OD copper tube (for carrier gas flow) in the "B" position of the Hewlett-Packard 5750 Research Gas Chromatograph. The carrier gas used was He, and compressed (Comp) air and hydrogen (H_2) were used for the H_2 -burner. The flow of the He carrier gas was measured at the exit port of the TC detectors with a 10 ml bubble tube and a stop watch. Since the splitter ratio was 1:1, the flow through the column was approximated by multiplying the measured flow (or calculated flow rate) by two. The H_2 flow was found by difference, from the He plus the H_2 flow at

the tips of the burners (with detector heaters off) minus the He flow at the tips of the burners. The pressure gauge settings and the flow rates used are given in Table 4.

TABLE 4
PRESSURE GAUGE SETTINGS AND FLOW RATES OF HELIUM,
COMPRESSED AIR AND HYDROGEN

Gases	Pressure Gauge Setting (lbs/in ²)	Flow Rate (ml/min)
He	40	108 ^a
Comp Air	34	500 ^b
H ₂	16	46 (50 ^b)

^aAverage of 23 measured flows on 11 different days

^bFrom Figure 4-1-3, page 4-1-5, Reference No. 99

Temperature operating range of GC and FI detector principles.

The dual FI detector was used for the development of the chromatograms with a splitter ratio of 1:1 with the TC detector. The FI detector is in the order of 1,000 times more sensitive than the TC detector and for most injections the only response by the TC detector was that of the solvent.

The temperature operating range used with the gas chromatograph over a period of about six weeks is given in Table 5. Even though the TC detector was not used for response in this study, the temperature had to be kept above that of the oven to insure the elution of the compounds through the detector splitter and out the TC detector exit port for collection. The bridge current reading corresponding to the temperature of the TC detector in Table 5 was 175-176 milliamperes.

TABLE 5
 TEMPERATURE (°C) OPERATING RANGE USED
 WITH THE GAS CHROMATOGRAPH

	Injection of Carbon Black Extract	Desorption With Pyrolyzer
Injection port	343-352	152-154
Oven	220-221	220-221
FI detector	320-325	323-324
TC detector	312-315	312-314
TC Oven	302-307	302-307
Auxiliary	306-312	309-312

The functioning electrical parts of the FI detector are the jet (tip of burner) and collector ring electrode. The H₂-line joins the He carrier gas line below the base of the detector and both pass through the jet of the burner. The Comp air flows through a cylindrical diffuse washer at the base of the burner and unites with the H₂ above the jet to form the H₂-flame, when lit. The organic material or compounds are partially ionized by the high energy H₂-flame after being eluted from the column. A battery supplies a potential between the collector ring and jet of the burner, which attracts the positive ions and electrons to oppositely charged poles. The collector ring and jet are biased so either can be positive with respect to ground. A voltage drop across the input load resistor, caused by the flowing collected electrons, is amplified by the electrometer (as explained previously, p. 35) and the output is shown on the potentio-

metric recorder as a peak.

The response of the ionization detector depends on the number of molecules per unit time entering the detector. It is not a direct function of the concentration of these molecules in the carrier gas. . . . (99)

which makes the FI detector principles different from those of the TC detector. The sensitivity of the FI detector (itself) can be increased by higher gas flow rates, to a limit. A higher H₂ flow rate causes a higher temperature and thus a greater percentage of ionization. Also, the ratio of H₂ to carrier gas flow rates will affect the sensitivity and the Comp air flow rate must be above a certain minimum for higher sensitivities. Normally, the detector response is plotted vs. the flow of one gas while holding the other flows constant, repeating the response vs. the flow of another gas, while holding the others constant, etc. for the three gases used.

The injected qualitative Std solutions of the Polyc Ar HCs and the extracted carbon black in CS₂ ranged in volume from 4.0 to 10.0 μ l in size. Two and 10 μ l Hamilton syringes were used for all injections. The solvent for the anthracene Std and the 40 μ g/ml B[a]P solution was i-C₈ rather than CS₂.

Desorption capillary tubes and pyrolyzer unit. The glass capillaries used for the small samples of carbon black for the desorption experiments in the pyrolyzer probe were plain Yankee Micro-Hematocrit Tubes from Clay-Adams, Inc., with original length of 75 mm and ID of 1.1-1.2 mm with wall thickness of 0.2 ± 0.02 mm. After washing thoroughly, including rinsing with benzene, the capillary tubes were dried and both ends sealed (to keep tubes clean plus the

warming of about 1 in of the open end before sealing it prevented a large glass bead on the end). The sealed capillary tube was cut, both lengths measured (to keep them separate) and each weighed. Then a carbon black sample was tamped into each tube to a length of about 7 mm (from sealed end), and the tubes plus carbon black reweighed (usually weighed two, etc. at a time). The tubes containing the carbon black were dried in the oven at a temperature of 105-110° C for 2 hrs, and cut off to a length of about 1.3 cm before being placed in the probe (some stored in a desiccator over anhydrous CaCl_2 until used). The first few capillaries containing carbon black did not have a plug of glass wool in them, but it was found that a shorter capillary (8-9 mm) with a small plug (about 1.5 mm) of glass wool in it was much cleaner in placing the probe in and taking it out of the injection port. Silanized glass wool from Analabs, Inc. was used after it had been washed with both benzene and $i\text{-C}_8$ and dried.

A semi-automatic Model 80 Pyrolyzer Unit with a spiral probe (0.125 in, OD, x 6 in, overall length) from F & M Scientific Corporation was used. This pyrolyzer consisted of a control cabinet with a current dial (for ammeter setting), a probe assembly and an interconnecting cable (102). Pictures of these units are given in the Manual and in Figures 8 and 9 by Thompson (102, 36).

The control cabinet has a main power switch and a selector switch so two different temperatures could be used with different current settings or the same approximate temperature could be used with two different probes. It also has a manual-automatic switch. The manual is used to set the current for the desired temperature, and after

once set, the automatic position is used. After the automatic switch was pushed, there was a delay for about a minute and then the electrode of the spiral probe was heated (or current passed) for about 15 sec.

Normally a pyrolyzer is used for the thermal decomposition of nonvolatile material which is not vaporized by the temperature of the injection port of a gas chromatograph (up to about 500° C). The Manual for the Pyrolyzer 80 Unit gives the temperature range of 800-1200° C, but most of the temperatures used in this study ranged from about 660 to 800° C (102). The calibration curve for the spiral probe used is given in Figure 4.

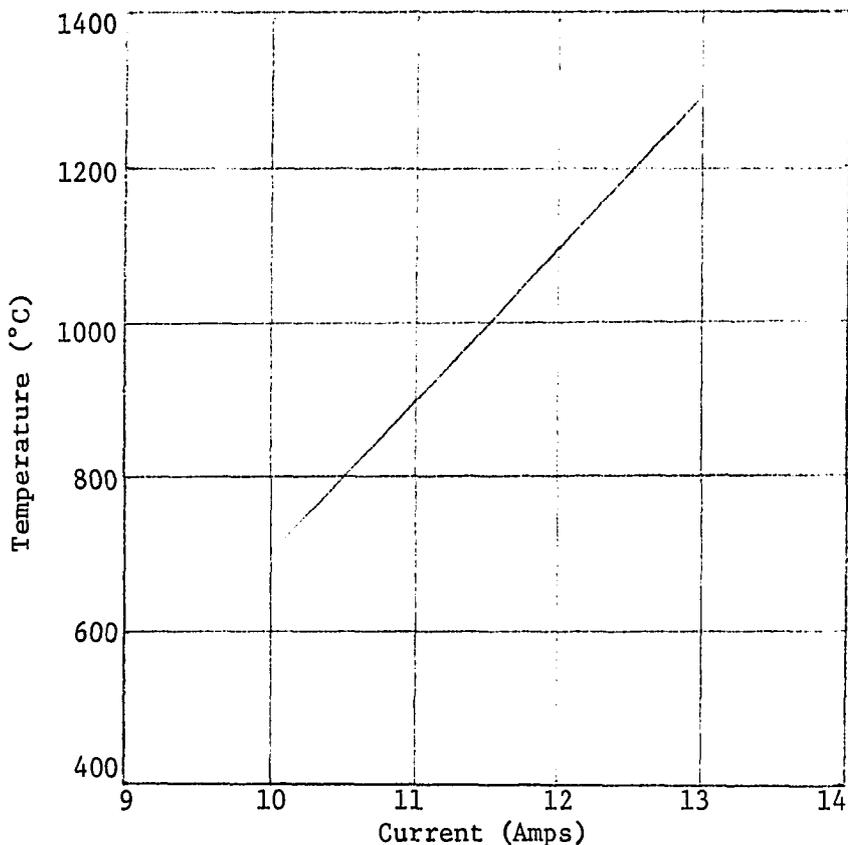


Fig. 4. Calibration curve for spiral probe at Helium flow rate of 60 ml/min.

The temperature range can only be approximated from this curve since it was calibrated for a He flow rate of 60 ml/min (compared to 108 used) and the line would have to be extrapolated at the lower temperature used. The current reading was 9.8 to 10.0 amperes, which gave a temperature used of about 660-700° C from Figure 4.

Collection of injected pyrene and B[a]P Stds from GC. In the early part of this study, several small injections (<2.0 μ l) of the B[a]P Std solution (40 μ g/ml in i-C₈) were made into the 24 ft, SE-52 column and the eluted peaks (not solvent) were collected in one U-tube containing i-C₈ chilled in an ice-water bath. The i-C₈ solution was concentrated to about 0.1 ml for a microcell and identification was attempted by an UV absorption spectrum with a Beckman DK-2A double beam spectrophotometer. Another prepared B[a]P Std in i-C₈ did not give the peak which was collected from each of the previous injections. The peak collected must have been due to some unidentified contaminate in the sample and later work implied that the t_R of B[a]P would have been much greater than that of the peak collected. With a splitter ratio of 1:1, it is also believed the detection limit of B[a]P with the FI detector was not reached by these small size injections. Sawicki (66) gives a detection limit of 5 μ g for B[a]P with a FI detector in GC work.

Two injections of the pyrene Std (40 μ g/ml) and two of B[a]P Std solution (60 μ g/ml) were made (separately) into the 4 ft x 0.125 in, SS, SE-30 column and the eluted respective peaks were collected in two small capillary tubes (74, 103). These were thin-walled borosilicate glass, open ended mp capillary tubes, 10 cm x about 1.75 mm, OD from

Fisher Scientific Co. One end of each tube was heated in a small flame and pulled slightly with forceps to give a smaller diameter end, which was wrapped with Teflon tape to make a tight seal when held into the exit port of the TC detector (since FI detector destroys the sample). A blank was collected for an equivalent time after the two injections of B[a]P and before the peaks were eluted. Similarly (as above), an injection of a 20 $\mu\text{g}/\text{ml}$ B[a]P Std solution was made and the eluted peak collected in a fourth capillary tube. These four collected samples were rinsed from the capillary tubes into separate small volumetric flasks with $i\text{-C}_8$ using a 1 ml syringe. They were then transported in an ice pack to the Environmental Health Department for examination with the SPF. The capillary tubes for all further peak collections (except three or four) were from 8.5 to 9.6 cm in length and contained a small plug (1-2 cm in length) of loosely packed glass wool (silanized). Three eluted components were collected in a glass wool plug in a 5 or 6 in Teflon tube of 0.125 in, ID and 0.25 in, OD.

Spectrophotofluorometer and Accessory Units

The SPF used was an Aminco-Bowman No. 4-8203 with an off-axis ellipsoidal mirror condensing system with xenon lamp and a 1P28 photomultiplier tube. The following accessory units were used with it: a xenon lamp power supply, Model No. 28153-1 made by Englehard Hanovia, Inc.; a solid state, blank subtract photomultiplier microphotometer, No. 10-280 from American Instrument Co., Inc. (Aminco); and a variable range X-Y recorder, Model No. 814A, made by Bolt Beranck and Newman, Inc.

Xenon lamp power supply. The xenon lamp power supply reduces

the fluctuating alternating current from about 0.25 per cent to 0.1 per cent and thus reduces the xenon pulsing and arc wander (104).

Photomultiplier microphotometer. The photomultiplier microphotometer has the following metermultiplier (MM) settings with a range of 1,000: 100, 30, 10, 3, 1, 0.3 and 0.1; with sensitivity control of 0 to 100 per cent on each MM setting (104). The 0.1 setting is the most sensitive with 100 the least. The microphotometer has the following other controls or switches: zero adjust, blank adjust switch and control, blank polarity switch and high voltage control.

Recorder. The X-Y Recorder was especially designed for spectrofluorometric work and plots wavelength vs. emission intensity on 8.5 in x 11 in chart paper (104). The X-axis (horizontal) scale factor is 2.4 volts full scale with a suppression of 1.0 volt, direct current. This SPF has an output over the range of 200 to 800 nm and is recorded on the X-axis.

The scale factor on the Y-axis (vertical) is 50 millivolts full scale with a ± 10 per cent variable adjustment and the accuracy is ± 0.2 per cent full scale. The Y-axis full scale deflection corresponds with full scale deflection on the photomultiplier microphotometer.

Cell, slit settings and monochromatic light path. The cell used was a fused quartz tube, 7 mm, ID x 40 mm high, requiring a minimum volume of 0.6 ml and also a cell adapter. The Ex and Fl spectra recorded in this study were uncorrected with the following slit settings: A = 1, B = 0, C = 1, D = 1, E = 0 and F = 1 mm.

An Aminco bulletin states:

During operation with the fluorescence cell, light from the xenon lamp is dispersed by the excitation monochromator (grating type) into monochromatic radiation incident on the sample. A similar monochromator disperses the fluorescent light from the sample into monochromatic radiation incident on the photomultiplier tube. The radiation is there transformed into a weak electrical signal and fed to the photometer where it is amplified (104).

Then the photometer output is indicated on the dial of the microphotometer and can be transmitted to the vertical axis of the recorder.

Ex and Fl spectra of Polyc Ar HC Std solutions. After the SPF accessory equipment had warmed up for about 15 min with the high voltage setting on the photomultiplier microphotometer on 700 volts, the power supply and xenon lamp were turned on. The pyrene Std solution in i-C₈ (about 1 ml in cell) was scanned with the Fl monochromator by hand at one or several (if necessary) Ex wavelength setting(s) to find any fluorescent bands (at a low sensitive setting to start with). The wavelengths at which Fl occurred were noted, and then with this (one or more) setting(s) for the Fl monochromator, the Ex monochromator was scanned by hand and any Ex peaks noted. After the main Ex and Fl peaks had been found for pyrene, a family of Fl curves was recorded by setting the Ex monochromator and scanning the wavelength region above this setting and similarly for any further Ex settings. Likewise a family of Ex curves was also recorded for each Fl setting by scanning the wavelength region lower than the Fl setting. Or as given in the Bulletin, a Fl spectrum is recorded (wavelength vs. intensity) when the recorder X-input is connected to the output of the scanning emission monochromator for a maximum Ex wavelength setting of the Ex monochromator (104).

Conversely, when the X-input of the recorder is connected to the output of the scanning Ex monochromator for a maximum Fl wavelength setting of the emission monochromator, an Ex spectrum is recorded (wavelength vs. intensity). An Ex and Fl spectra of a pyrene solution are depicted by the dashed and solid lines, respectively, in Figure 5. The maximum Ex and Fl wavelength settings for the pyrene Std were found to be 332 and 381 nm, respectively.

This same procedure was repeated for the B[a]P Std solution and the other Polyc Ar HC Stds in i-C₈ and from the family of curves for each compound, the best (maximum in most cases) Ex and Fl wavelength setting was determined.

Confirmation of collected pyrene and B[a]P peaks from GC. The previous collected pyrene peak from the GC in i-C₈ was examined spectrophotofluorometrically with the Fl spectrum recorded at an Ex wavelength of 332 nm and the Ex spectrum recorded at a Fl wavelength setting of 381 nm (Figure 5).

Similarly, the collected peaks from the B[a]P injections into the gas chromatograph were examined with the SPF. The Ex and Fl wavelength settings for the Fl and Ex spectra of B[a]P were 380 and 402 nm, respectively. Both the collected GC peaks of pyrene and B[a]P were confirmed by comparing Ex and Fl spectra to those of Std solutions.

Collection of GC Peaks from Carbon Black for SPF Examination

Carbon black extract. After confirming the identity of the collected GC peaks of the injected pyrene and B[a]P Std solutions, 12 peaks from the gas chromatographic separation of injected samples of

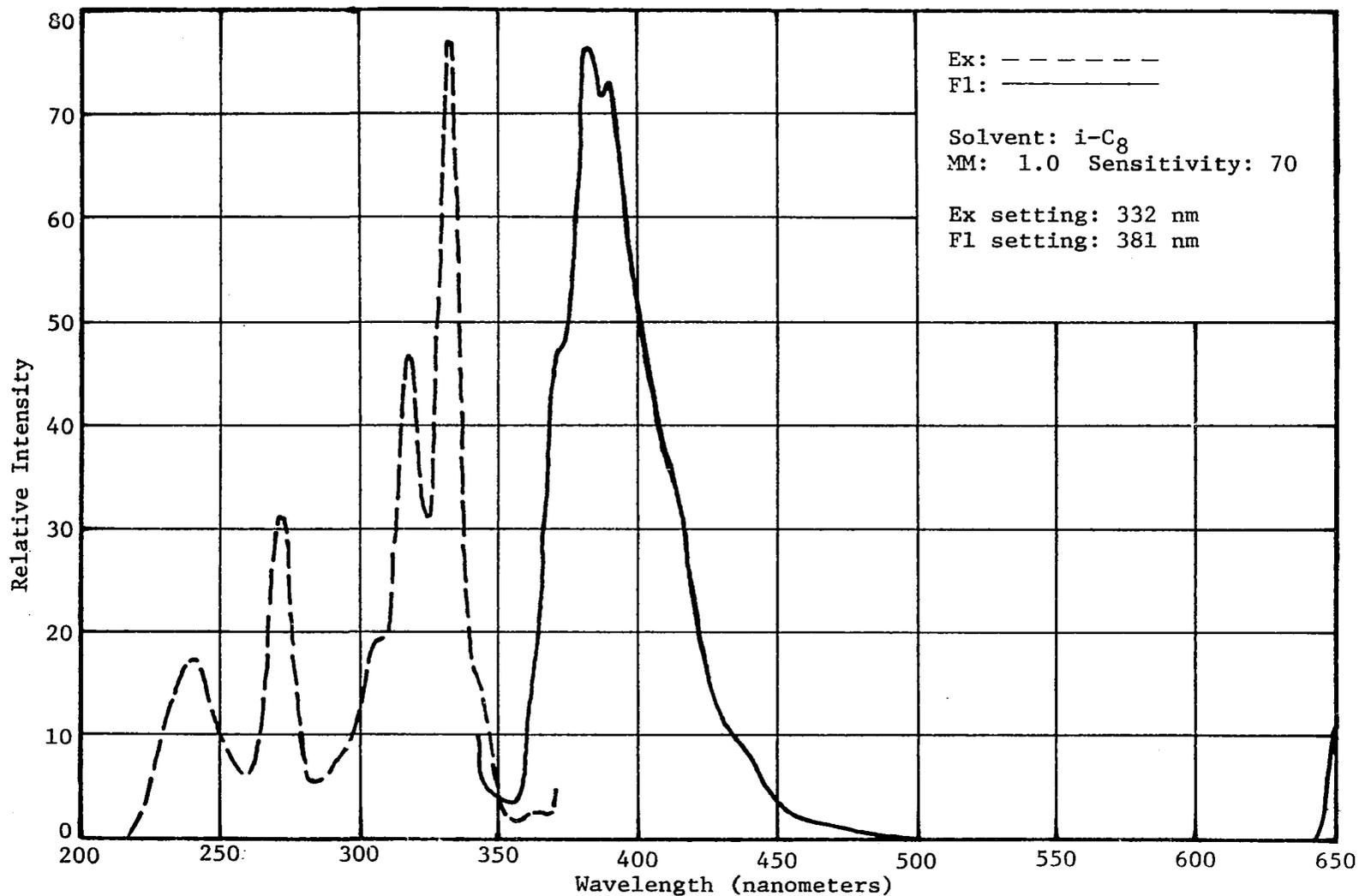


Fig. 5. Excitation and fluorescence spectra of chromatographic peak from injection of pyrene standard solution.

the carbon black extract were collected and examined with the SPF. After finding that some of the collected peaks contained more than one compound, the approximate front and back half of peak 10 and peak 11 were collected for fluorometric examination.

Carbon black desorption experiment. A chromatogram of the Polyc Ar HCs desorbed from a carbon black sample in a capillary tube was compared to that of the injected carbon black extract. In one interesting desorption experiment with carbon black in a capillary tube of 1.3 cm in length, the latter two or three peaks were not present. Peaks 8 and 10 were collected from this particular chromatogram for spectrophotofluorometric examination. On close examination of the capillary tube when taking it and the probe out of the injection port, a reddish-brown band was seen on the opposite side of the glass wool plug from the carbon black. This material had a reddish fluorescent color under a UV lamp, and was stored in a N₂ atmosphere in a 5 in screw capped pyrex test tube in the refrigerator. Later the end with the reddish band was cut off, dissolved in i-C₈ and examined with the SPF. Since the fluorescent intensity of Anthran in it was so strong, the i-C₈ solution was concentrated to about 0.2 ml and streaked on a 7.75 in x 22.5 in Whatman No. 1 filter paper. Two small drops of the following Polyc Ar HC Stds were placed in line with the streak (same distance from beginning end): B[a]P, B[ghi]Pery, Anthran and coronene. The paper was dipped in 35 per cent N,N-dimethylformamide in ether (vol/vol) just up to the applied streak (by capillary action) and developed for 4 hrs with descending i-C₈ (52). The paper chromatogram was allowed to dry in the dark room for about 10 minutes after

taking out of the tank, was examined under the Chromato-Vue UV lamp and five fluorescent bands marked. Each band was cut out, cut up into small chips and eluted five times with warm methanol with filtration through a fine sintered glass funnel using N₂ pressure. The methanol solution of each band in a 50 ml Erlenmeyer flask was evaporated to about 0.2 ml while on the steam bath and a N₂ jet, the flask rinsed down two times with about 5 ml of benzene and three more times with about 5 ml of i-C₈, with evaporation to a small volume after each rinse. The final volume was adjusted to about 1 ml and each of the five fractions in i-C₈ was examined with the SPF.

In a later desorption experiment of the carbon black in a shorter capillary tube (8 mm) with the pyrolyzer, the latter peaks were present on the chromatogram. Six peaks from this experiment were collected and examined with the SPF. Peak 8 was compared to that of the carbon black extract, since no Std Polyc Ar HC of this peak was available at the time.

CHAPTER IV

RESULTS AND DISCUSSION

Polyc Ar HC t_R and Gas Chromatogram of Carbon Black Extract

The t_R may be defined as the time along the baseline (or abscissa) from the injection point to the perpendicular line dropped from the top of an eluted peak of a given compound (100).

The average t_{RS} (in min) of the ten Polyc Ar HCs used in this study were determined on the 4 ft x 0.25 in, SS, SE-30 column and are given in Table 6. The number of injections used to determine this average and the concentration of each HC is included in this table. In most cases both pyrene and B[a]P Stds were injected into the gas chromatograph to check their t_{RS} prior to injections of the carbon black extract or the carbon black desorption experiment. This was done in addition to measuring the He carrier flow at the exit ports of the TC detector.

Injections of the carbon black extract in CS_2 were made at the isothermal oven conditions of 200, 220, and 230° C to determine the better temperature for chromatogram development. The peak containing B[a]P (Peak 10) had a t_R of about 35 min at 200° C and about 10 min at 230° C. The response (peak height) was poor and there

TABLE 6

CONCENTRATION, NUMBER OF INJECTIONS AND AVERAGE RETENTION TIME
OF POLYCYCLIC AROMATIC HYDROCARBONS

Polyc Ar HC	Concentration ($\mu\text{g/ml}$)	No. of Injections	Average t_R (min)
Anthracene	40	2	1.1
F	40	3	2.1
Pyrene	40	13	2.34
B[a]Anth	60	2	5.38
Chrysene	60	2	5.42
B[e]P	45	2	13.9
B[a]P	60	10	14.5
B[ghi]Pery	48	2	35.3
Anthran	60	2	38.9
Coronene	61	1	84-85

was a great deal of tailing at the lower temperature, while response was better, but the resolution (separation of peaks) was poor at the higher temperature. The isothermal oven conditions of 220° C seemed to be a compromise between the response and resolution. It was not advisable to use temperature programming without dual columns and under the conditions of the desorption experiments (low injection port temperature).

The developed gas chromatogram of the carbon black extract is shown by the dashed line in Figure 6. The injected 6.0 μl of CS_2 solution corresponds to a wt of 2.2 mg of carbon black. The peaks which

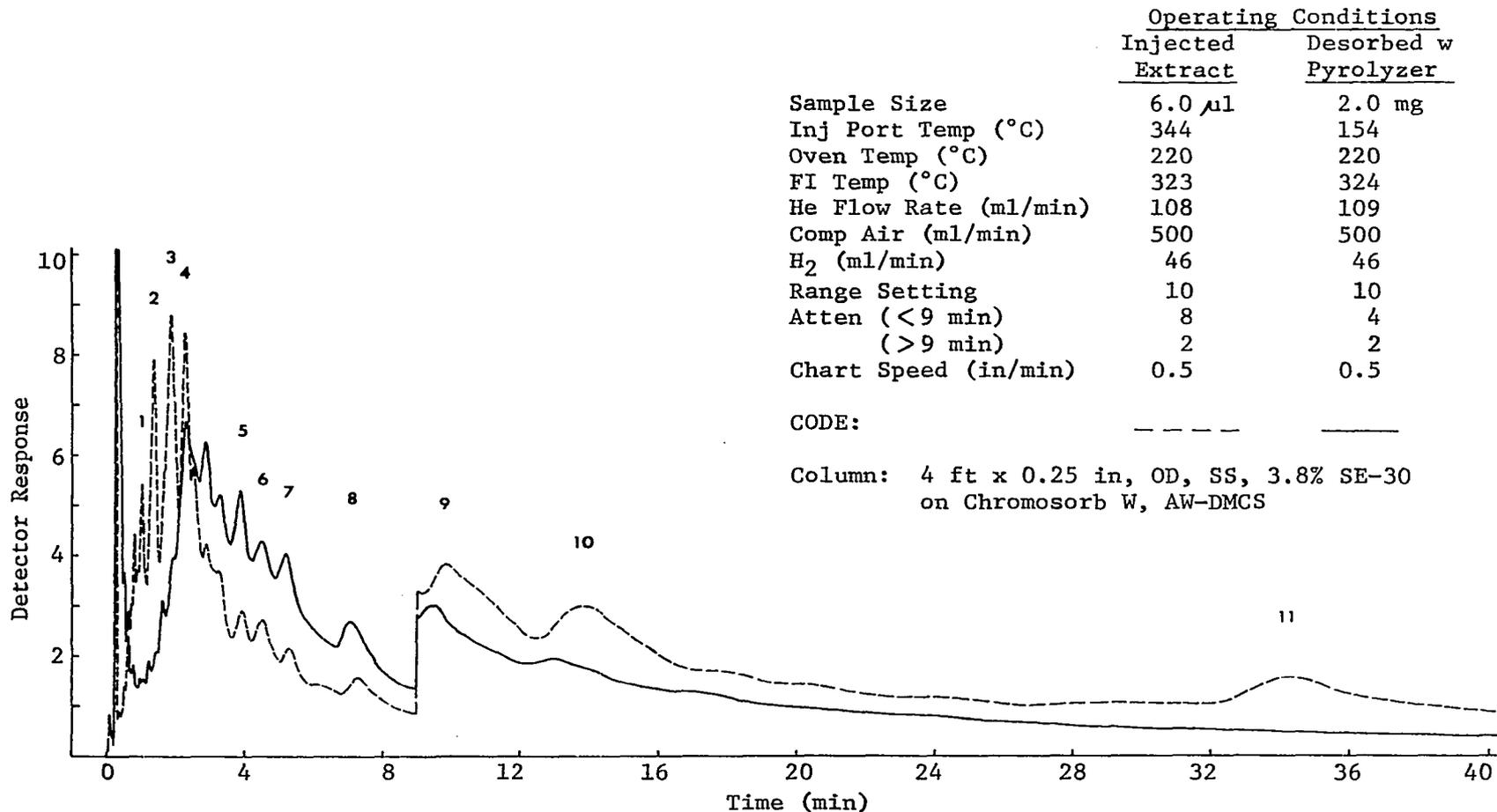


Fig. 6. Gas chromatograms of polycyclic aromatic hydrocarbons from carbon black with flame ionization detector. Peak numbers correspond to the compounds listed in Table 7.

were collected for SPF examination from the chromatogram are also numbered in the Figure. Peak No. 12 had a retention time of about 84 min and was not clearly distinguishable on some chromatograms. Compound(s) in Peak Nos. 1, 3, 4, 7, 10, 11 and 12 were tentatively identified by their t_R .

Confirmation of Polyc Ar HCs in Chromatographic
Peaks of Carbon Black Extract

As previously given (p. 19), the confirmation of the compounds in these peaks is very analogous to that reported by Chakraborty and Long (78). They collected gas chromatographic peaks containing Polyc Ar HCs from soot samples and used the UV spectrophotometer for identification, while in this work the SPF was used. A comparison of the compounds collected from the chromatographic separation of the carbon black extract is made with those identified by Chakraborty and Long and is shown in Table 7. There was some question about the identity of Peak 7 (Chakraborty and Long's work) by Thomas and Monkman (105), but in this correspondence Long and Chakraborty (106) believed Peak 7 to be a mixture of 1- and 4-methylpyrene. Peak 9B was given as a pyrene derivative in the original work, but Wallcave (107) reported the compound to be cyclopenta[c,d]pyrene by mass spectroscopy.

Lijinsky and Mason (108) had previously reported the incomplete separation of some of these same Polyc Ar HCs from a coal tar extract by GC. These were: anthracene and phenanthrene; B[a]Anth and chrysene; B[j]F and B[k]F; B[e]P, B[a]P and perylene; and B[ghi]Pery and dibenz[a,h]anthracene.

As previously reported in this work, the approximate front and

back half of both Peak 10 and Peak 11 were collected separately (called 10A, 10B, and 11A and 11B) for fluorometric examination.

The identification of ten Polyc Ar HCs (given in Table 7) from the carbon black extract was confirmed by their Ex and Fl spectra. The Ex and Fl spectra of all these collected gas chromatographic peaks (except Peak 8), along with the Stds for those identified are given collectively in Appendix B. The Ex and Fl spectra of Peak 10A were compared to both the spectra of B[e]P and B[a]P Stds and the spectra of Peak 10B were compared to those of the B[a]P Std. The Ex and Fl spectra of Peaks 11A and 11B were compared to the spectra of B[ghi]Pery and Anthan Stds, respectively.

Chromatogram, Collection of Peaks and Identification
of Polyc Ar HCs in Desorption Experiment

The gas chromatogram of the carbon black desorption experiment in a 1.3 cm length capillary tube with attached pyrolyzer is shown by the solid line in Figure 6. The similarity of the two chromatograms can be seen. A possible explanation of the lower peak heights of the first 2 min in the desorption chromatogram is that the capillary tube in the probe was in the injection port at about 150° C for 2 hrs, resulting in the partial volatilization of the lower boiling components before the pyrolyzer was fired. The very high boiling Polyc Ar HCs were not driven out of the capillary tube, which would explain the non-appearance of the latter two or three peaks.

There was no question about Anthan being one of the compounds in the reddish fluorescent material from the spectrophotofluorometric examination of the i-C₈ solution in which the small capillary end was

TABLE 7

COMPARISON OF COMPOUNDS COLLECTED FROM CHROMATOGRAPHIC SEPARATION OF
CARBON BLACK EXTRACT TO IDENTIFIED COMPOUNDS FROM SOOT SAMPLES

Peak No.	Compound(s) Identified (or Unidentified)	Compounds Identified by C. & L. ^a	C. & L. ^a Peak No.
1	Anthracene	Anthracene Phenanthrene	3
2	Unidentified	Methylphenanthrenes	4
3	F	F	5
4	Pyrene	Pyrene	6
5	Unidentified	1- and 4-Methylpyrene	7
6	Unidentified	Benzo[mno]fluoranthene	8
7	B[a]Anth Chrysene	B[a]Anth Chrysene	9A
8	Unidentified	Cyclopenta[c,d]pyrene	9B
9	Unidentified	B[b]F and B[k]F	10
10A	B[e]P	B[e]P	11
10B	B[a]P	B[a]P Perylene	
		Indeno[1,2,3-cd]pyrene	12
11A	B[ghi]Pery	B[ghi]Pery	13
11B	Anthran	Anthran	14
12	Coronene	Coronene	15

^aChakraborty and Long

placed. Sawicki, Hauser and Stanley (64) reported that Anthan can be identified in a complicated mixture of about 25 HCs. After the paper chromatogram was run on this i-C₈ solution, B[ghi]Pery and coronene were identified in the second band (from origin). The Ex spectrum for B[ghi]Pery was more conclusive than the Fl spectrum, with the emission peaks matching those of the Std at 298, 361 and 380 nm. A greater percentage of Anthan was in band three, with a small amount in band four of the paper chromatogram. Band four was also examined spectrophotofluorometrically for B[a]Anth, chrysene and B[a]P, but no definite identification was made. However, the Ex spectrum for B[a]P did have emission at two wavelengths (360 and 380 nm) corresponding to that of the Std, but not of the proportionate intensity. Band five was examined for B[a]Anth, B[a]P and Anthan, with the B[a]P fluorescent spectra being similar to band four (peaks at about 411 and 428-433 nm). Band one from the paper chromatogram had a R_f about six-tenths of that for band two (with B[ghi]Pery and coronene). The compound(s) was not identified, but had Ex peaks at 325, 339, 403 and 425 nm when scanned at a Fl wavelength setting of 456 nm; and Fl peaks of 418, 456 and 487 nm when scanned at Ex wavelength setting of 325 nm.

From a later desorption experiment of carbon black in a shorter capillary tube (8 mm), gas chromatographic Peaks 7, 8, 10, 11A, 11B and 12 were collected and the i-C₈ solution of each examined with the SPF. The shorter capillary tube in the probe insured the removal of the higher boiling compounds from it into the column where they were separated chromatographically.

Peak 7 was collected during a t_R of 5.1-5.8 min. Figures 7

and 8 show the Ex and Fl spectra of Peak 7 compared to those of B[a]-Anth and chrysene Std solutions, respectively. It is fairly clear that these two Polyc Ar HCs are present in this Peak. Sawicki *et al.* (51) gave a Fl spectrum (in pentane) of chrysene with a chrysene fraction of air-borne particulates separated by column chromatog. The main emission peaks (at 362, 381 and 401 nm) are sharper for his pure chrysene than in the fraction, which was also observed in this work. Similarly, in both this work and that reported by Howard and co-workers (52, 54), the Ex and Fl emission spectra for the reference of B[a]Anth was sharper than that in the unknown. Interestingly, it was reported that B[a]Anth and chrysene give an identical Fl spectrum at an Ex setting of 284 nm (51).

Figure 9 compares the Ex and Fl spectra of the chromatographic Peak 8 from a desorption experiment (solid line) to that of Peak 8 from an injection of the carbon black extract (dashed line). It was originally thought that this Peak would be an ideal one to collect for identification, since it was more isolated and was thought to be one compound. It was collected during a t_R of 7.0 to 7.9 min. The Ex and Fl spectra of the pair appeared to be identical, but no definite conclusion was made concerning the presence of more than one compound in the peak.

Chromatographic Peak 10 was collected during t_R of 13.2 to 15.9 min and Figure 10 compares the Ex and Fl spectra of it in *i*-C₈ with that of the B[a]P Std solution. The presence of B[a]P in this peak could be confirmed from either the Ex or Fl spectrum, but both further substantiate its identity. The relative intensities of the Ex peaks at 295 nm (higher) and 360 and 380 nm (lower) are different

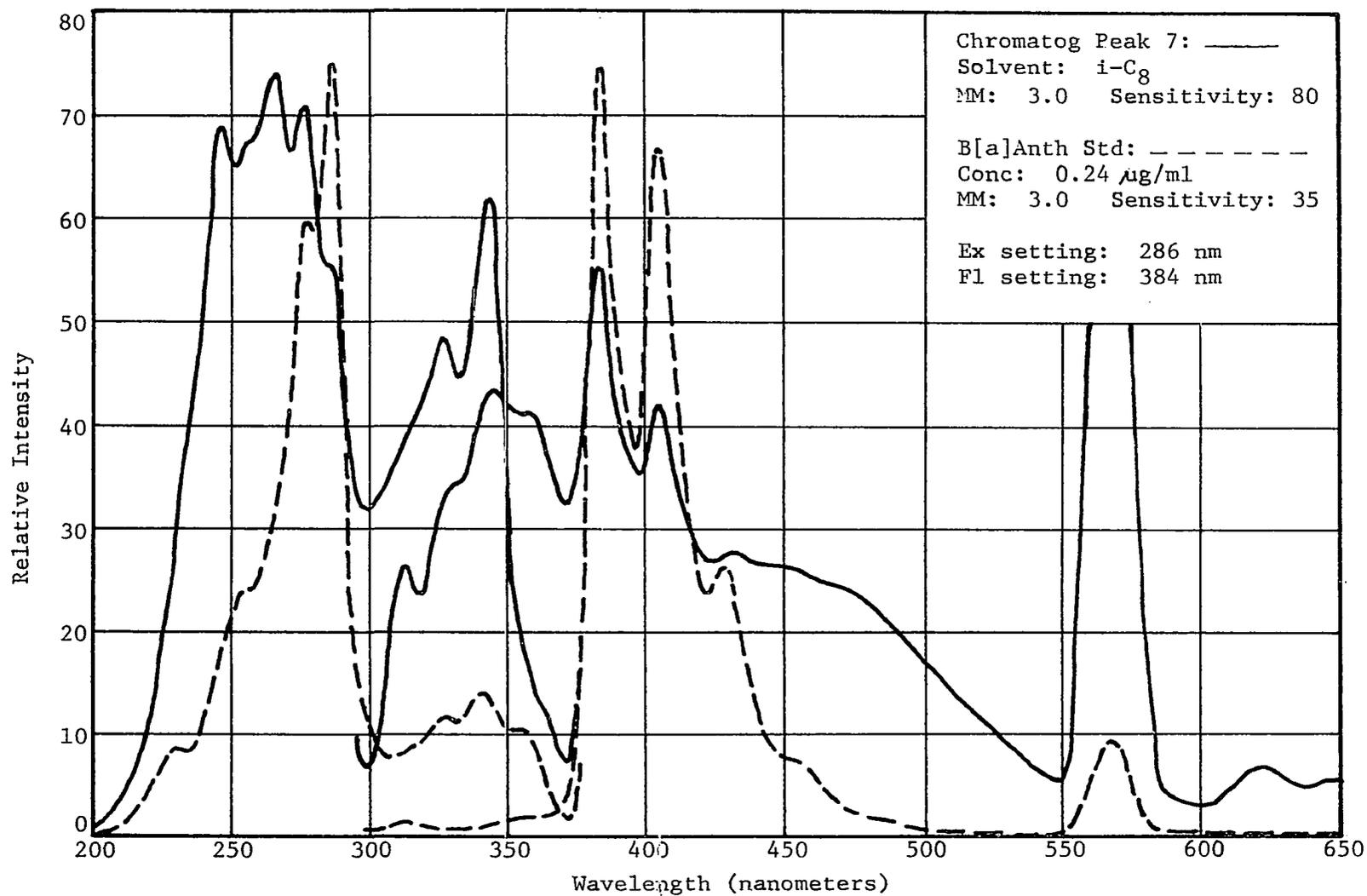


Fig. 7. Excitation and fluorescence spectra of chromatographic Peak 7 and benz[a]anthracene.

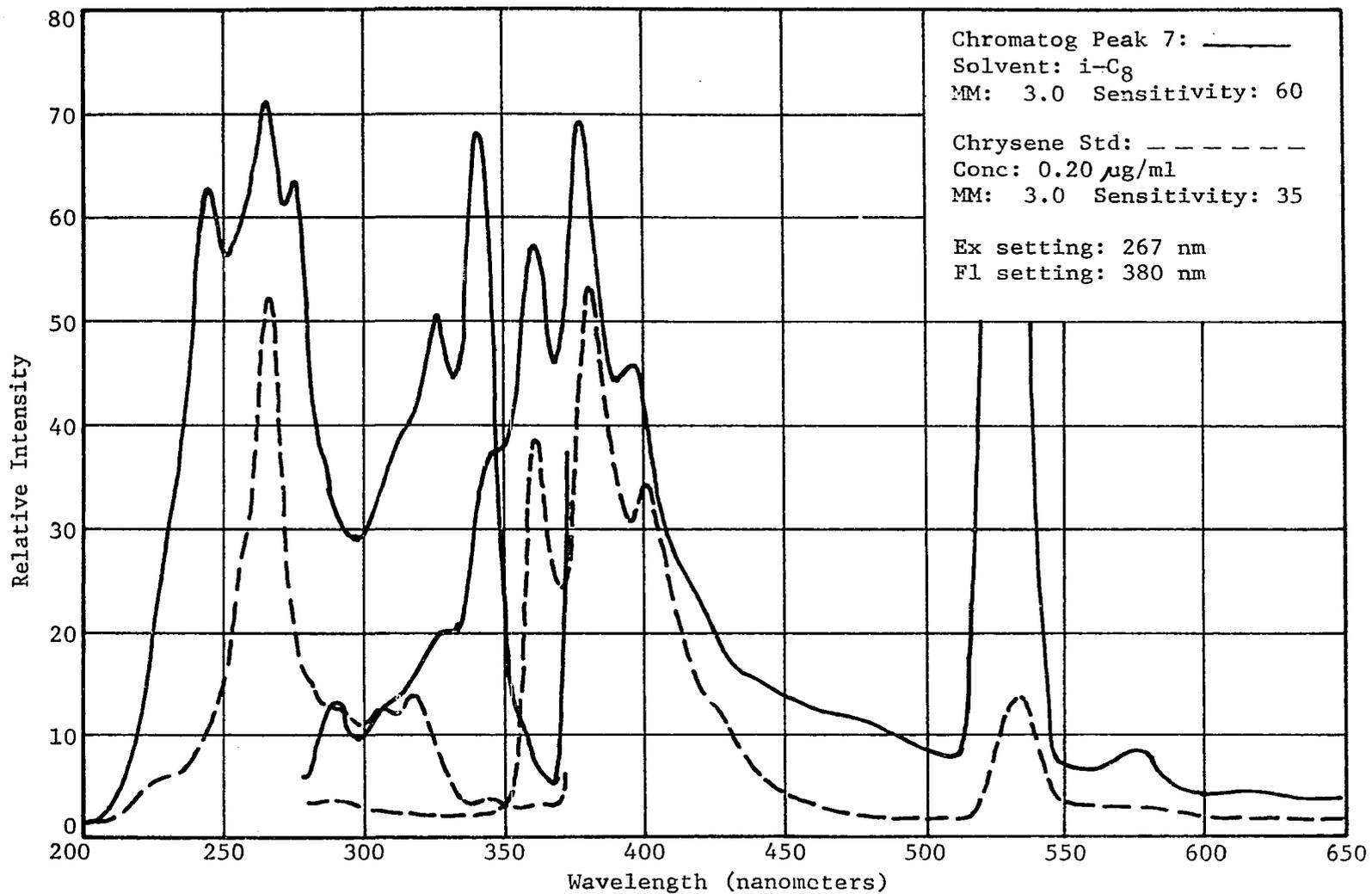


Fig. 8. Excitation and fluorescence spectra of chromatographic Peak 7 and chrysene.

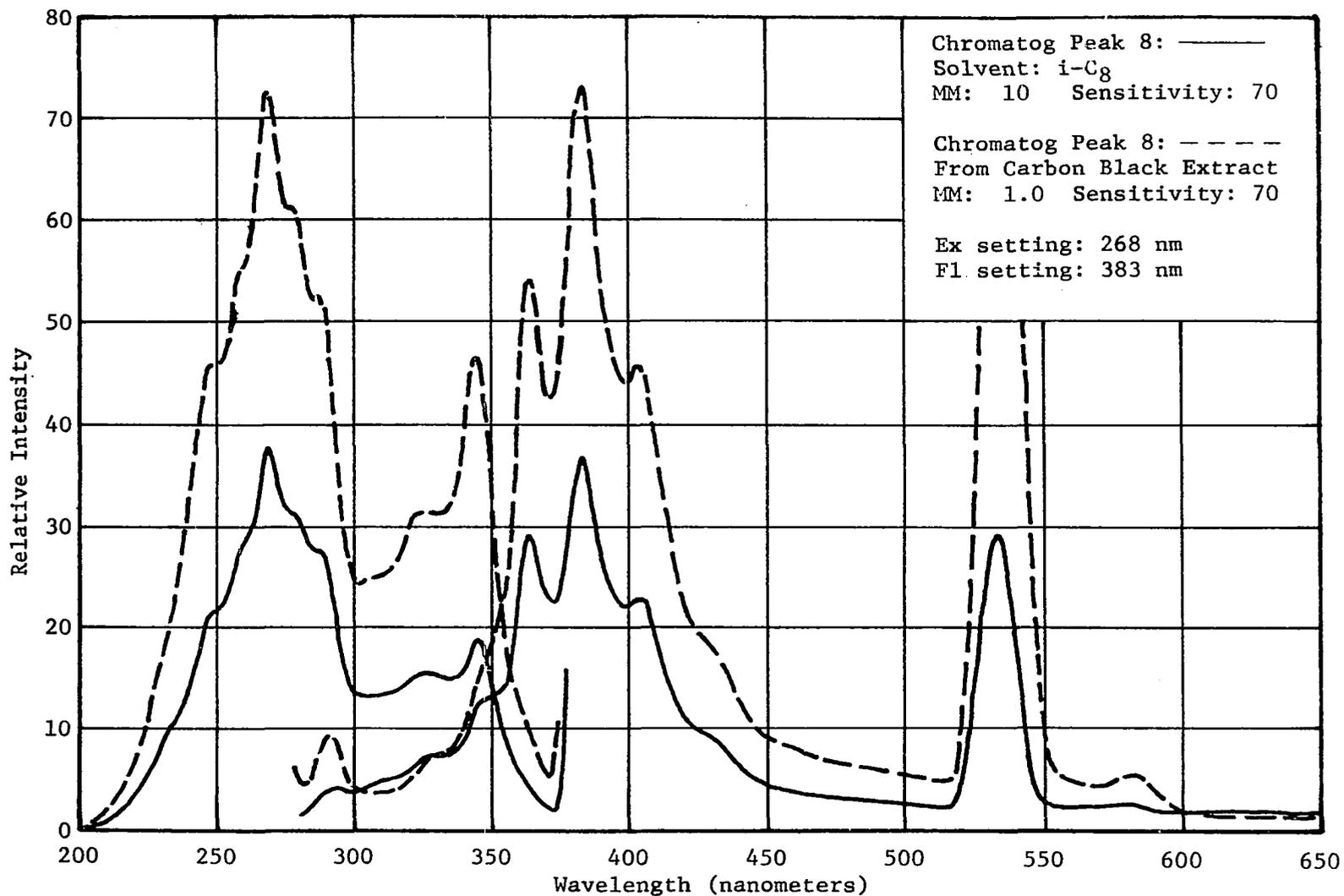


Fig. 9. Excitation and fluorescence spectra of chromatographic Peak 8 from carbon black extract and carbon black desorption.

from those of the corresponding peaks in the Ex spectrum of the Std. In a recent Talanta Review, Sawicki (109) reported that the appearance of new peaks or different relative intensities of known peaks in an Ex or Fl spectrum gave an indication that a solution contains more than one fluorescent compound. The broadening of the Fl peaks at 425 and 451 nm of the collected chromatographic Peak 10 gives an indication that there is a compound in this solution that has Fl peaks at about 430 and 455 nm. There is the possibility this broadening could be caused by some B[k]F in this solution, since Sawicki, Hauser and Stanley (64) have reported the Fl peaks of 400, 428 and 457 nm for B[k]F in pentane at an Ex wavelength setting of 302 nm. For comparison they used an Ex and Fl wavelength setting of 381 and 403 nm, respectively for B[a]P in pentane, while in this work 380 and 402 nm, respectively, were used in *i*-C₈. No B[k]F was available at the time this sample was examined, so a definite conclusion was not reached about the presence of B[k]F. Other unpublished quantitative work similar to Howard and co-workers' (52, 54) method of analysis has definitely shown the presence of B[a]P in this carbon black (by the UV spectrum).

Chromatographic Peak 10 was examined with the SPF for the presence of B[e]P, also. The spectra gave an indication that B[e]P was present in this solution, but it was not as conclusive as that for Peak 10A shown in Appendix B.

After the t_R of Std B[ghi]Pery and Anthran solutions were found to be different by about 4 min, two parts of Peak 11 were collected from both the carbon black extract and the desorption experiment.

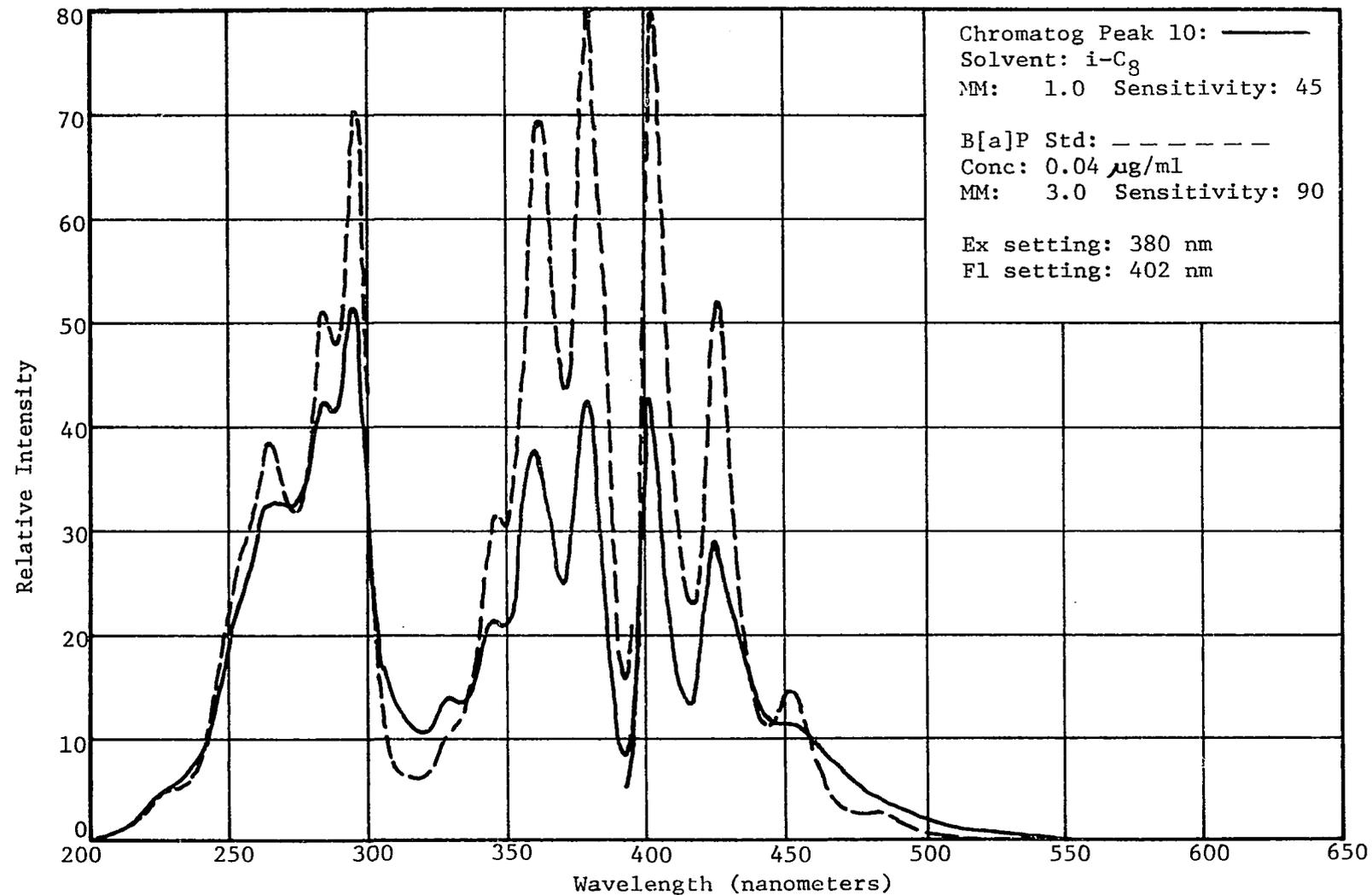


Fig. 10. Excitation and fluorescence spectra of chromatographic Peak 10 and benzo[a]pyrene.

In the desorption experiment, Peak 11A and 11B were collected from 33.5 to 36.8 min and 37.6 to 41.8 min, respectively. The spectra of these two collected peaks are compared with the spectra of B[ghi]Pery and Anthan Std solutions in Figures 11 and 12, respectively. The separation between the two compounds (as collected) was not entirely complete, but the identification of both B[ghi]Pery and Anthan was confirmed from their Ex and Fl spectra. The spectra shown for B[ghi]Pery is very similar to that reported by Howard and co-workers (52, 54). Anthan was also shown to be present in this carbon black by the UV absorption spectrum of a band separated by thin-layer chromatog as was B[a]P further identified (as above).

A collection at the exit port of the TC detector with a small capillary tube was made during a t_R of 80.8 to 85.8 min, even though Peak 12 was not entirely apparent (from the recorder). After the tube was rinsed out with $i-C_8$, the Ex and Fl spectrum was run with the SPF. It is represented by the solid line in Figure 13, while the dashed line is that of the coronene Std solution. The resolution of the Fl spectrum of this collected compound was better than that for the Std, which could be explained by replacing the xenon lamp with a new one and also using a higher voltage (800 volts) on the photomultiplier microphotometer. This spectra of coronene from the gas chromatograph is more nearly identical to that of the Std than was reported by Sawicki et al. (51) from a polluted air sample.

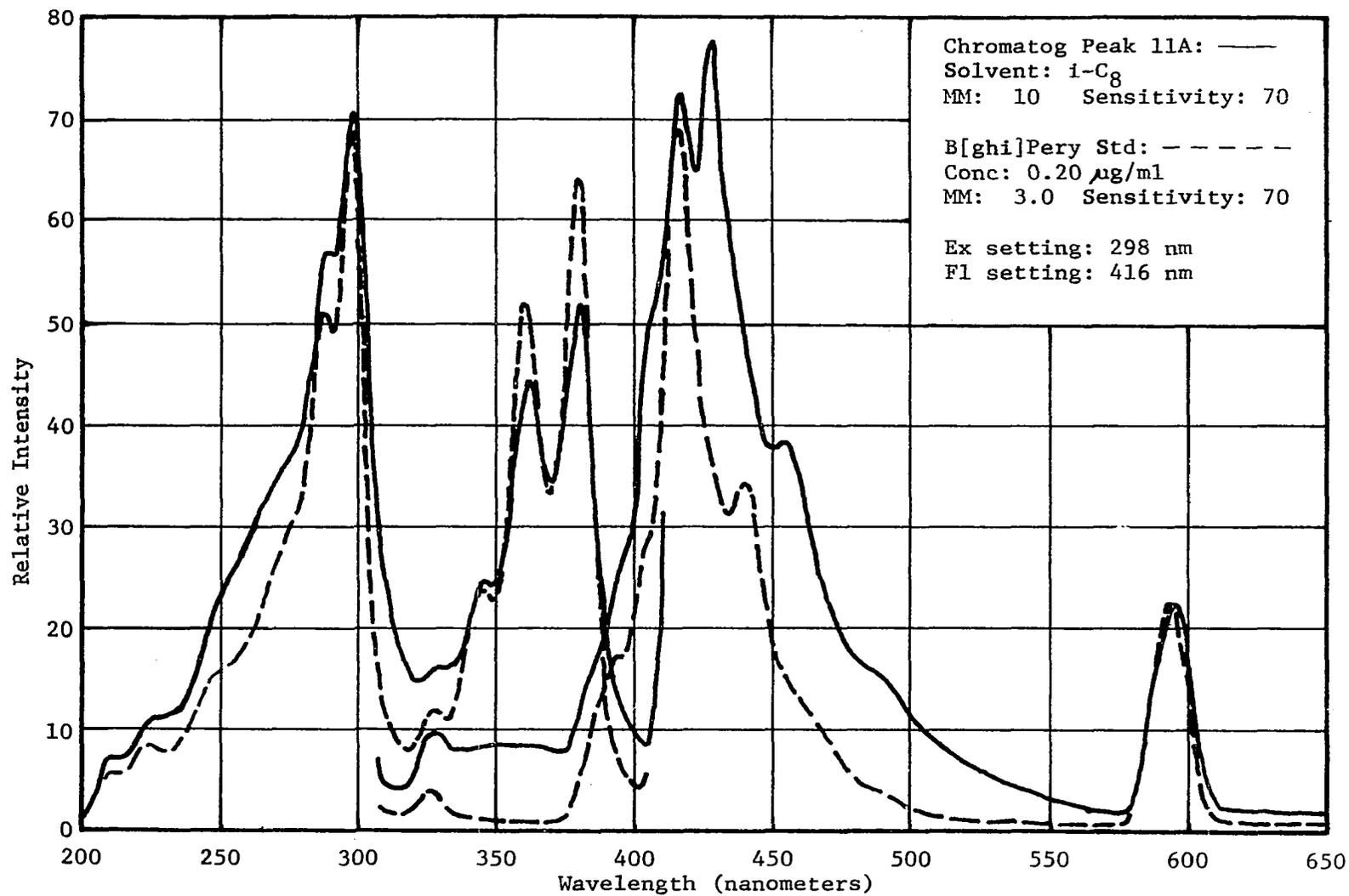


Fig. 11. Excitation and fluorescence spectra of chromatographic Peak 11A and benzo[ghi]perylene.

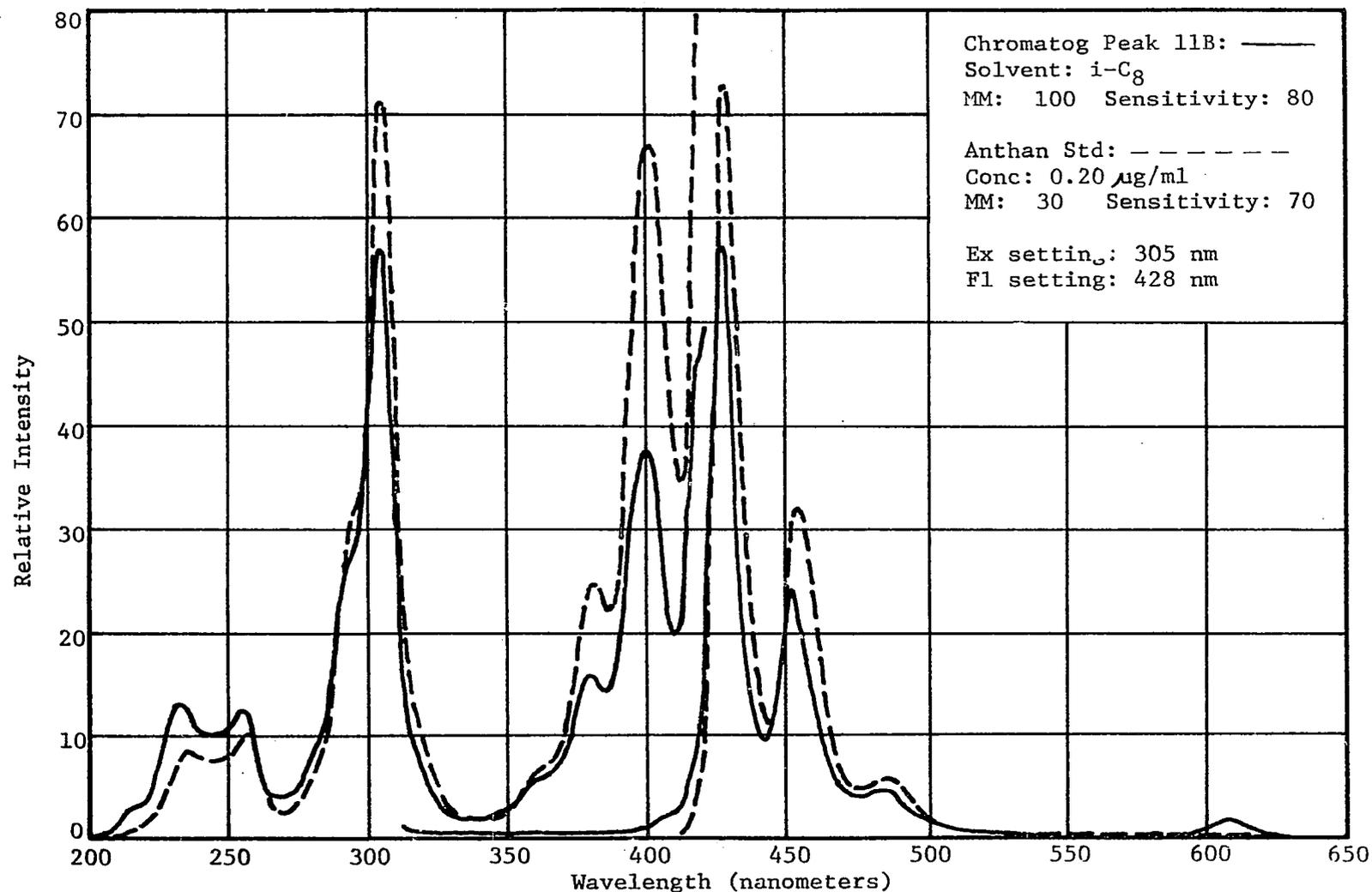


Fig. 12. Excitation and fluorescence spectra of chromatographic Peak 11B and anthanthrene.

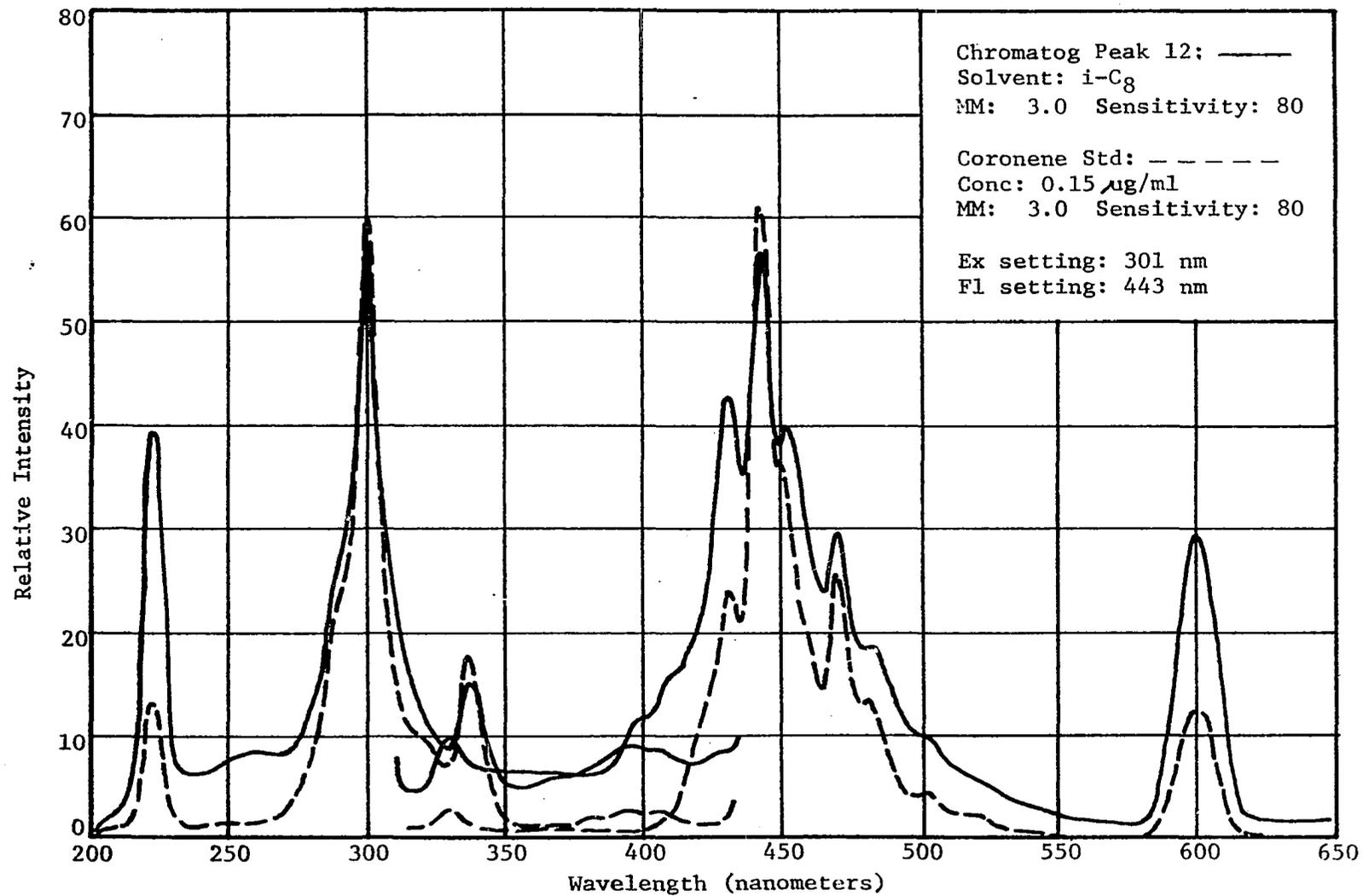


Fig. 13. Excitation and fluorescence spectra of chromatographic Peak 12 and coronene.

CHAPTER V

SUMMARY AND CONCLUSIONS

It has been known for about 20 years that both carbon black and rubber products contained Polyc Ar HCs and more recently (within the last 10 years) it has been found that these compounds are in the atmosphere in a range of a few parts per million. At least four of these Polyc Ar HCs from carbon black and/or rubber products and six from the air-borne particulates have been shown to be carcinogenic.

There has been a tremendous amount of research on both the carcinogenicity and the analysis of these compounds. Since most of the methods of analysis are long and tedious and because the Department of Environmental Health of the University of Oklahoma Medical Center is involved in research with carbon black and rubber dust, this study was undertaken. It also should be mentioned that the public health concern of these carcinogenic Polyc Ar HCs should not be taken lightly.

The identity of the Polyc Ar HCs in a fine furnace thermal carbon black was performed by two different procedures, namely: (a) the collected peaks from a gas chromatographic separation of the extract in CS₂ were examined with the SPF and the Ex and Fl spectra compared to those of qualitative Std solutions; and (b) the collected peaks from the gas chromatographic separation of the desorption of the Polyc Ar

HCs from the carbon black with an attached pyrolyzer were similarly analyzed with the SPF and the Ex and Fl spectra compared to those of Std solutions.

The following Polyc Ar HCs from the carbon black extract were tentatively identified by their t_R s on the 4 ft x 0.25 in, OD, 3.8 per cent SE-30, SS column and confirmed by their Ex and Fl spectra:

- a) anthracene
- b) F
- c) pyrene
- d) B[a]Anth
- e) chrysene
- f) B[e]P
- g) B[a]P
- h) B[ghi]Pery
- i) Anthan
- j) coronene.

In the desorption experiment of this carbon black, the identification of the following Polyc Ar HCs (with corresponding chromatographic peak number in parentheses) was confirmed by fluorometric examination:

- a) B[a]Anth (7)
- b) chrysene (7)
- c) B[a]P (10)
- d) B[ghi]Pery (11A)
- e) Anthan (11B)
- f) coronene (12).

In addition to the identification of these six compounds, the Ex and F1 spectra of Peak 8 from the gas chromatographic separation of the carbon black extract and the desorption experiment appeared to be identical.

The following conclusions are thus made from this study:

- 1) The Polyc Ar HCs can be desorbed from this carbon black.
- 2) The desorbed Polyc Ar HCs can be separated and tentatively identified by gas chromatographic methods.
- 3) The eluted chromatographic peaks can be collected and the compound(s) confirmed in these peaks (by SPF in this study).
- 4) It appears that a shorter, direct method of qualitative identification of the Polyc Ar HCs has been developed.

It is possible that the method is applicable to other types of samples, including air particulates and dust samples. The following improvements may be possible with this method:

- 1) The selection of columns which will completely resolve the compounds in the sample.
- 2) The use of the electron capture detector detector for increased sensitivity.
- 3) The use of a back flushing attachment for the very high boiling components.
- 4) The quantitation of a specific Polyc Ar HC or of a number of them.

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APPENDIX A

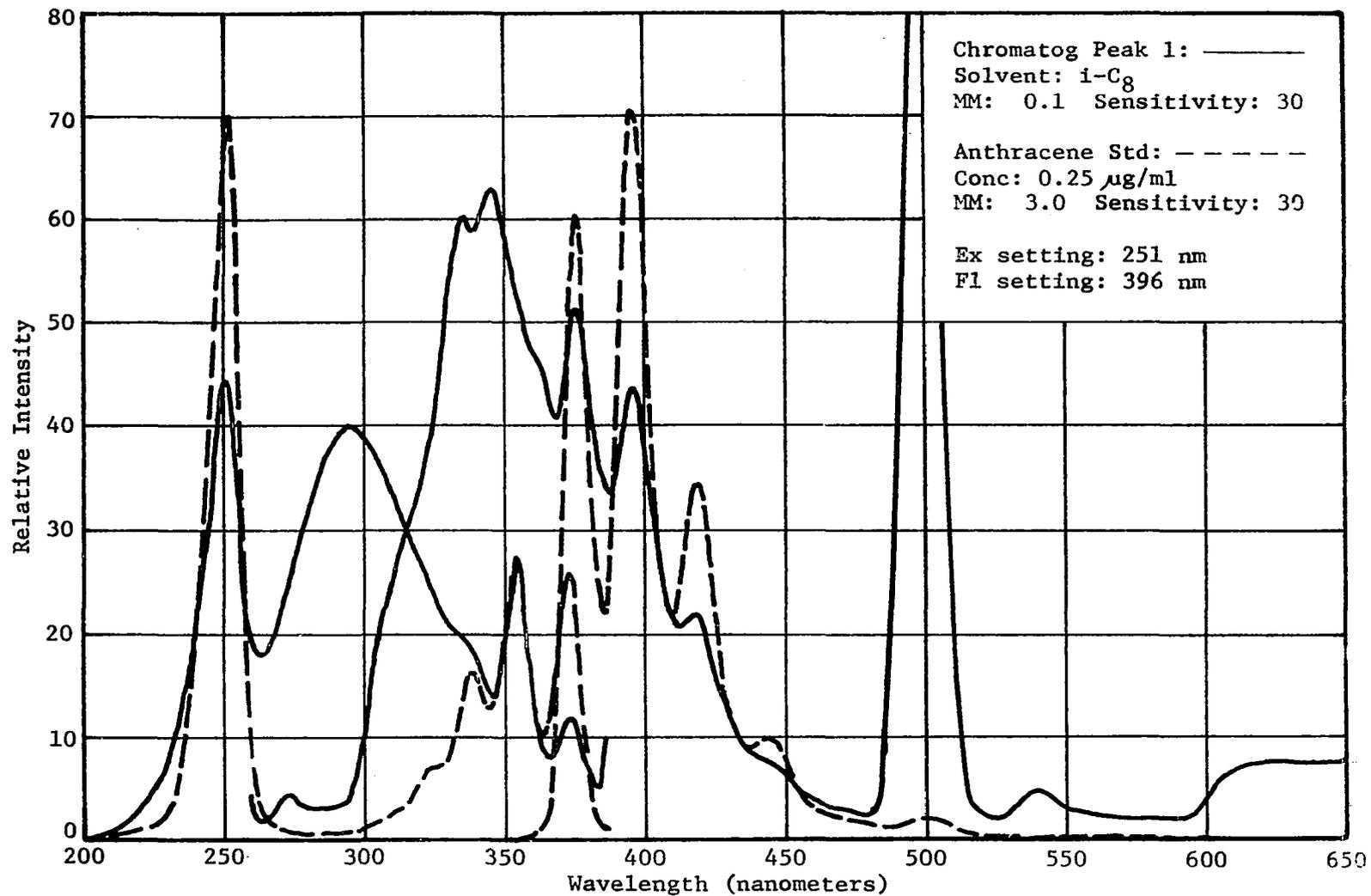
A list of abbreviations used in this text:

<u>Abbreviation</u>	<u>Name</u>
Anthran	anthanthrene
B[a]Anth	benz[a]anthracene
B[a]P	benzo[a]pyrene
B[e]P	benzo[e]pyrene
B[b]F	benzo[b]fluoranthene
B[j]F	benzo[j]fluoranthene
B[k]F	benzo[k]fluoranthene
B[ghi]Pery	benzo[ghi]perylene
F	fluoranthene
CS ₂	carbon disulfide
chromatog	chromatography
Comp	compressed
Ex	excitation
FI	flame ionization
Fl	fluorescence
GC	gas chromatography
i-C ₈	isooctane
MM	metermultiplier
μg	microgram
μl	microliter
mol wt	molecular weight
ng	nanogram

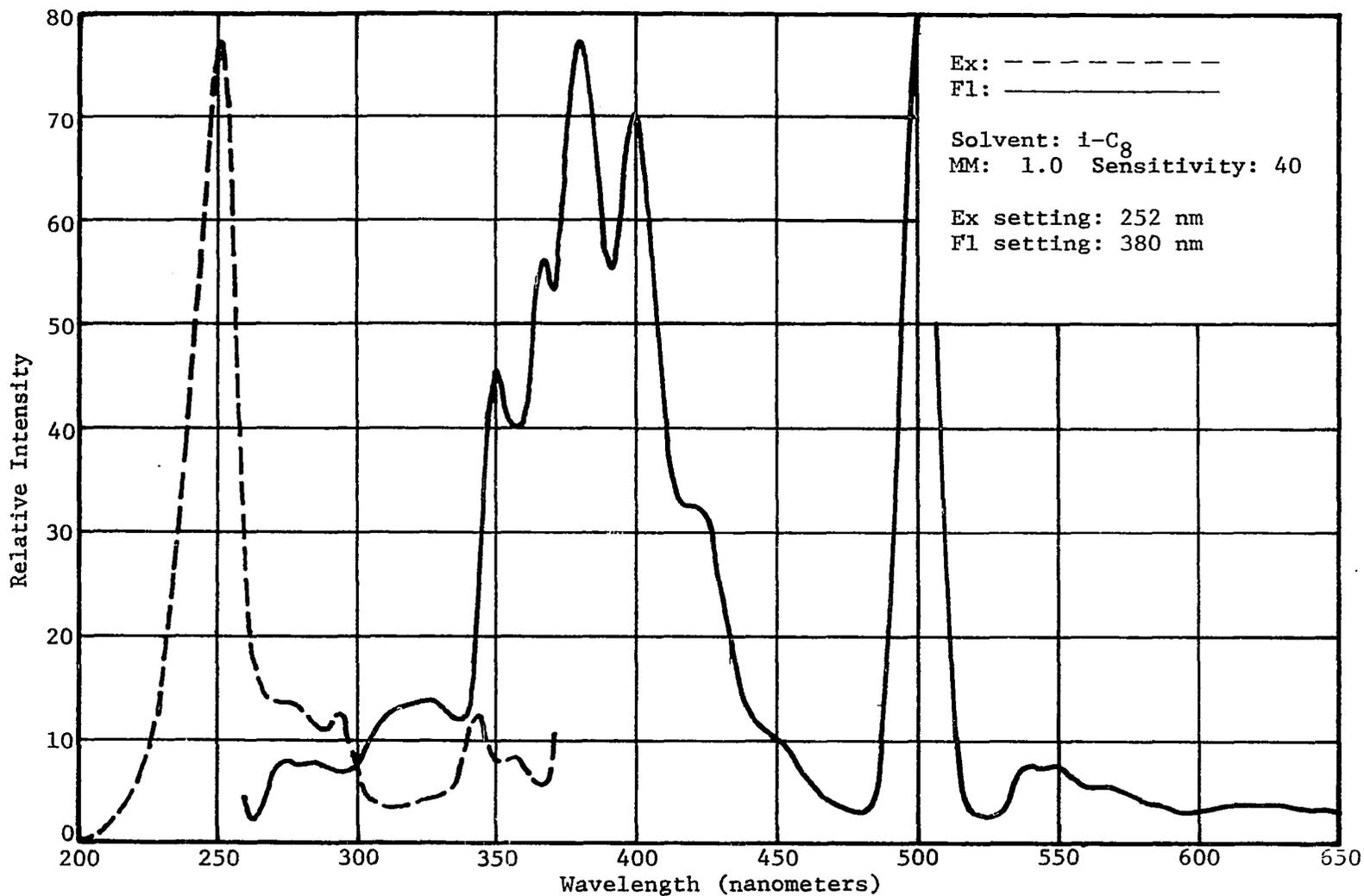
Abbreviations - Continued

<u>Abbreviation</u>	<u>Name</u>
nm	nanometer (same as millicron unit)
Polyc Ar HCs	polycyclic aromatic hydrocarbons
SPF	spectrophotofluorometer
SS	stainless steel
Std	standard
TC	thermal conductivity
t_R	retention time
UV	ultraviolet

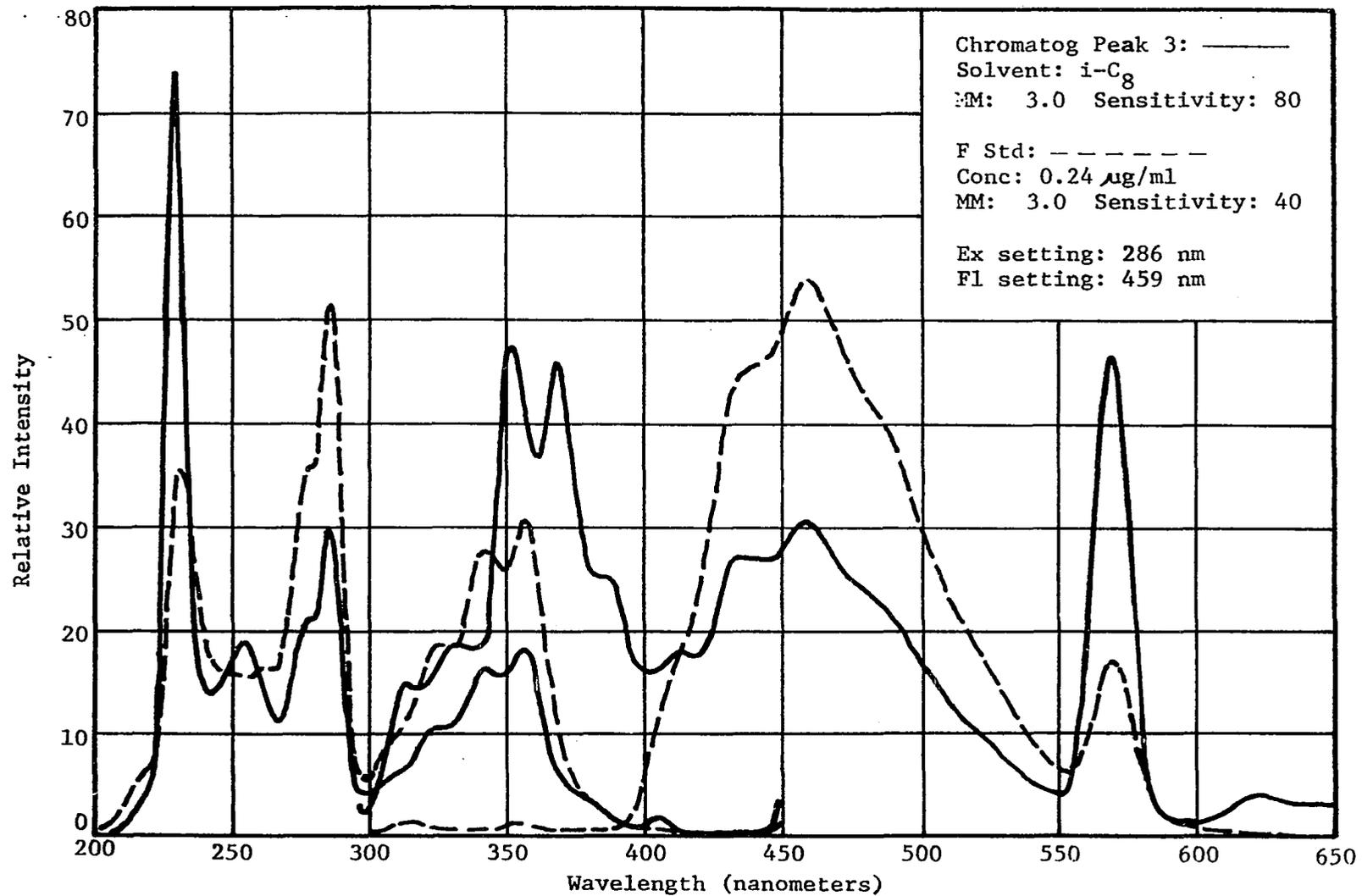
APPENDIX B



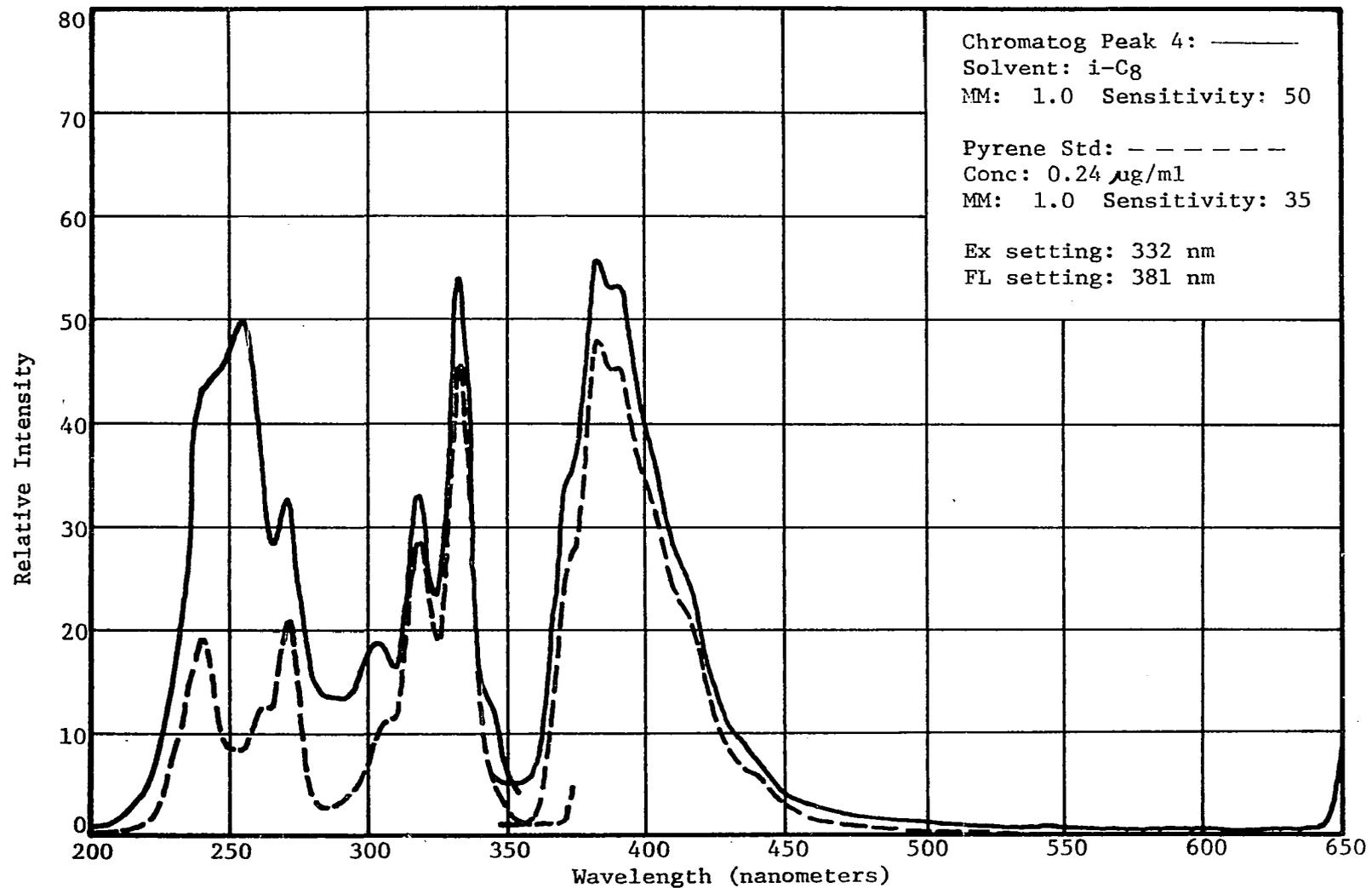
Excitation and fluorescence spectra of chromatographic Peak 1 from carbon black extract and anthracene.



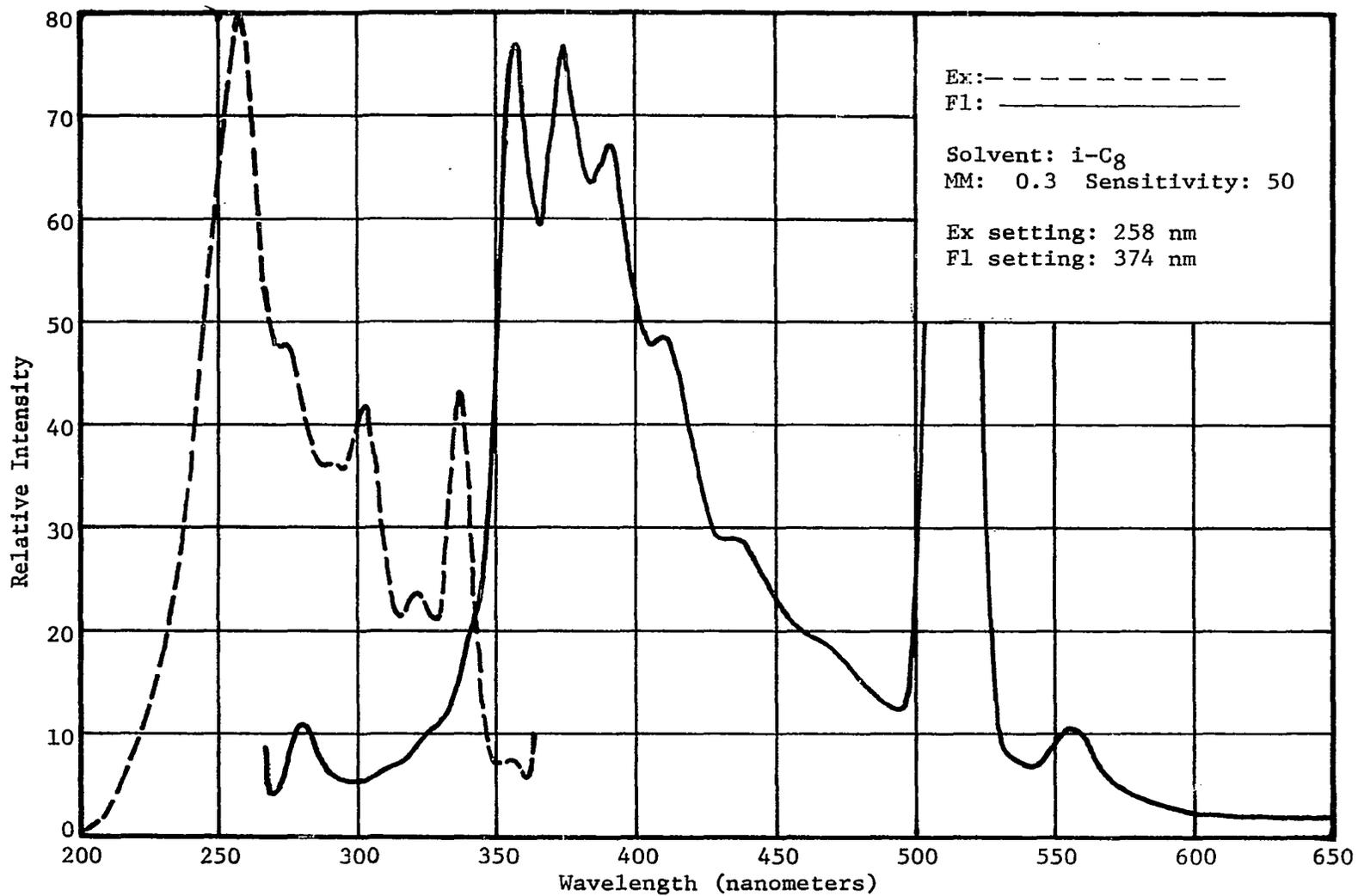
Excitation and fluorescence spectra of chromatographic Peak 2 from carbon black extract.



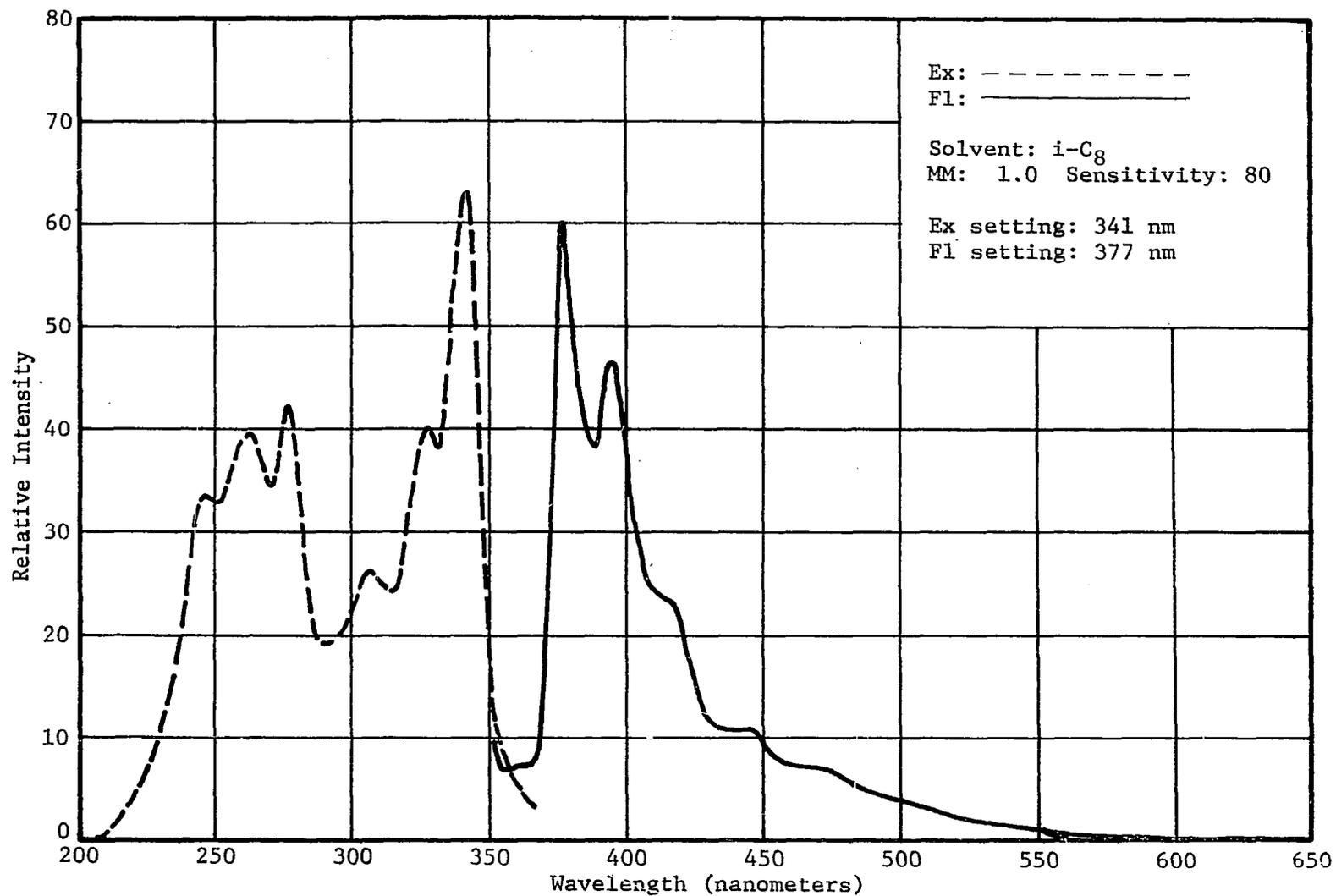
Excitation and fluorescence spectra of chromatographic Peak 3 from carbon black extract and fluoranthene.



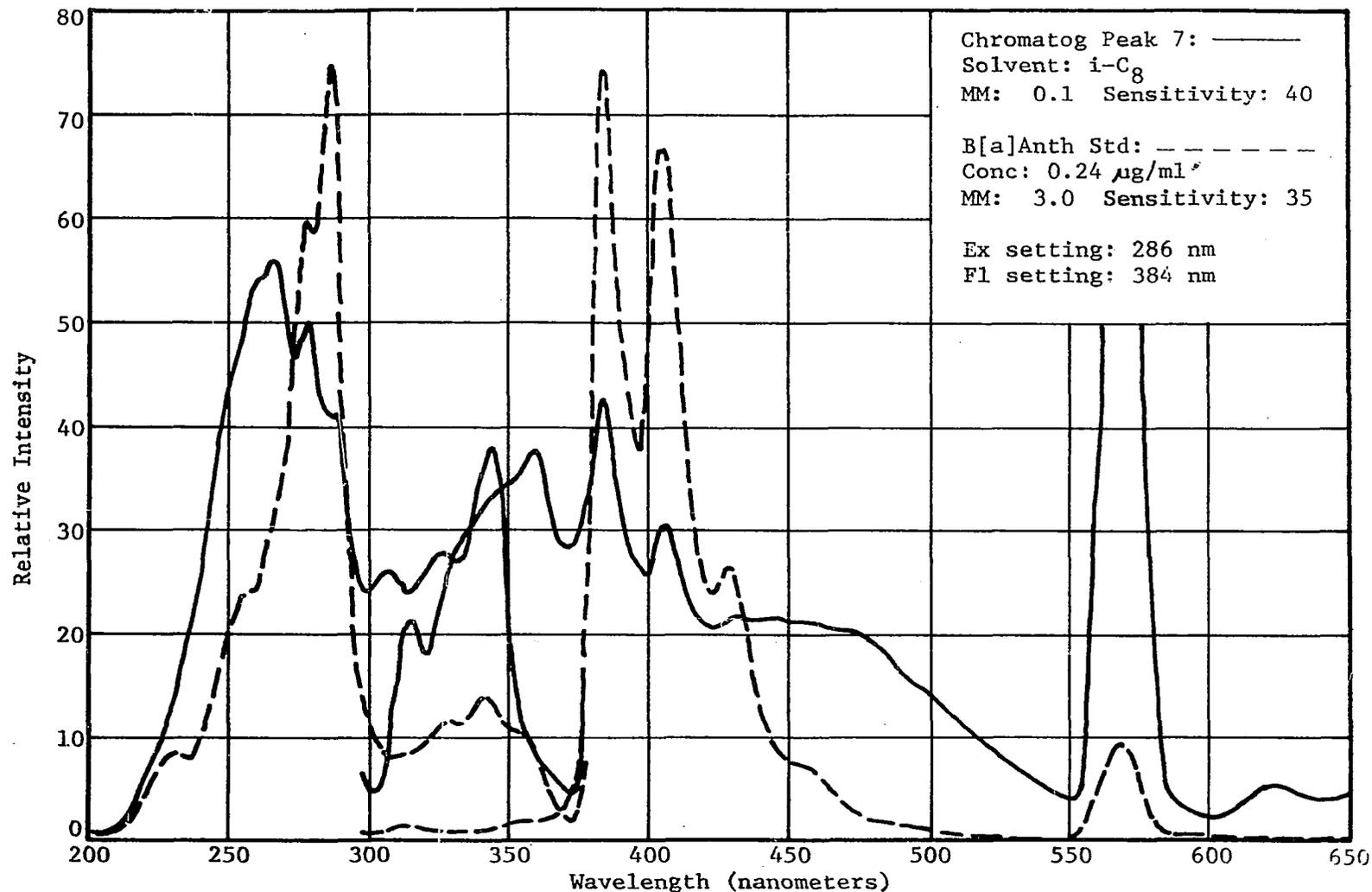
Excitation and fluorescence spectra of chromatographic Peak 4 from carbon black extract and pyrene.



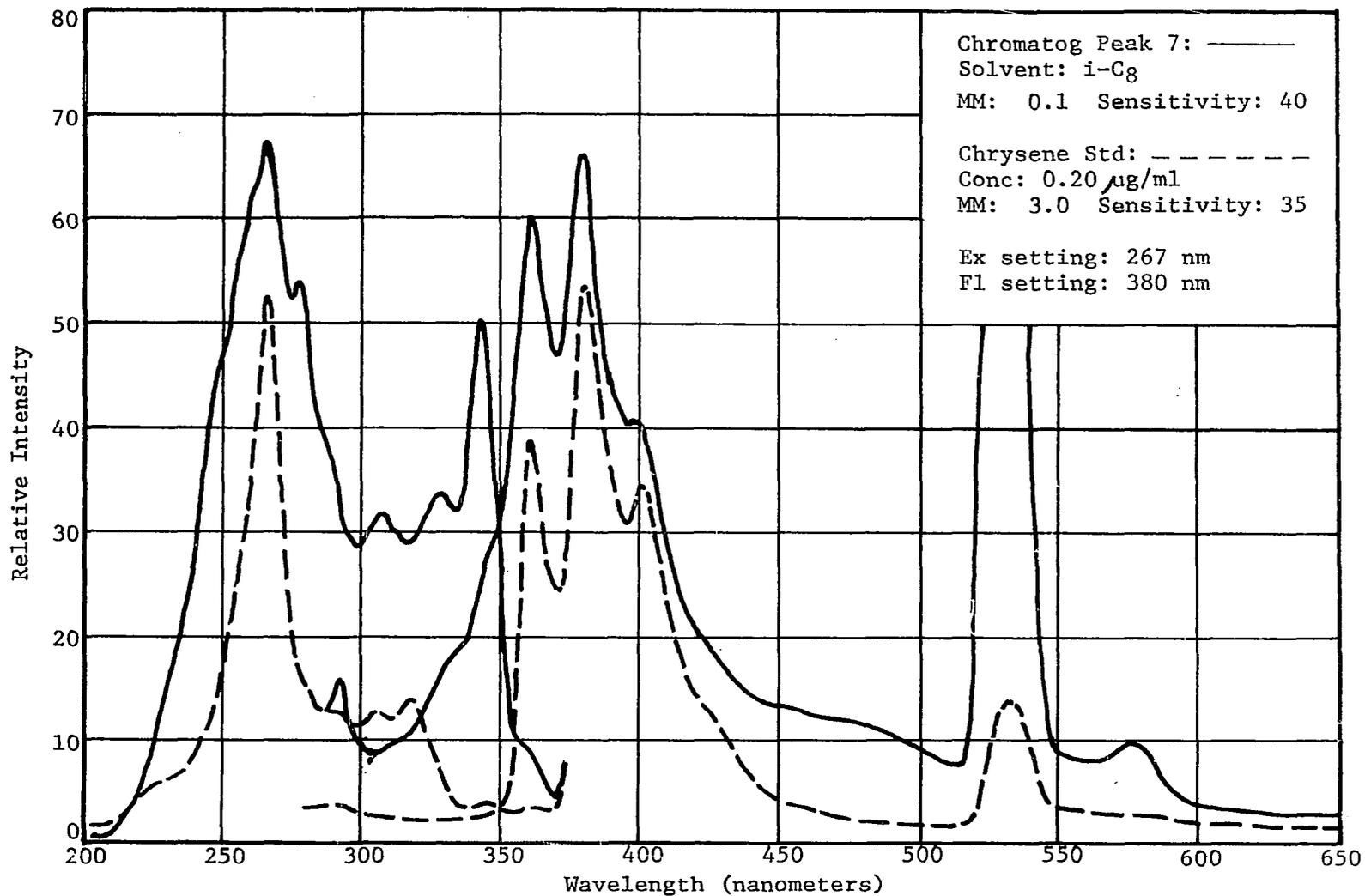
Excitation and fluorescence spectra of chromatographic Peak 5 from carbon black extract.



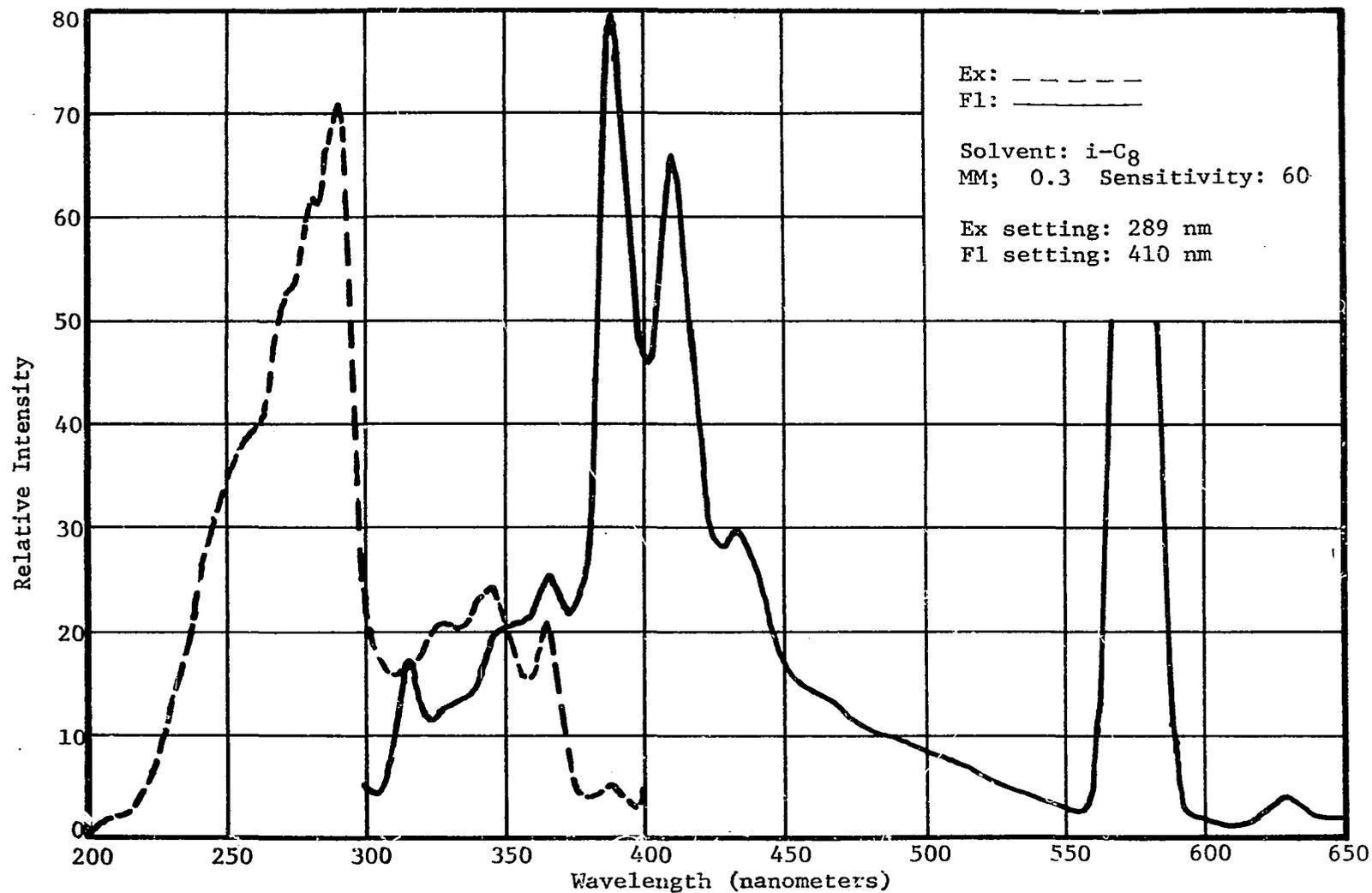
Excitation and fluorescence spectra of chromatographic Peak 6 from carbon black extract.



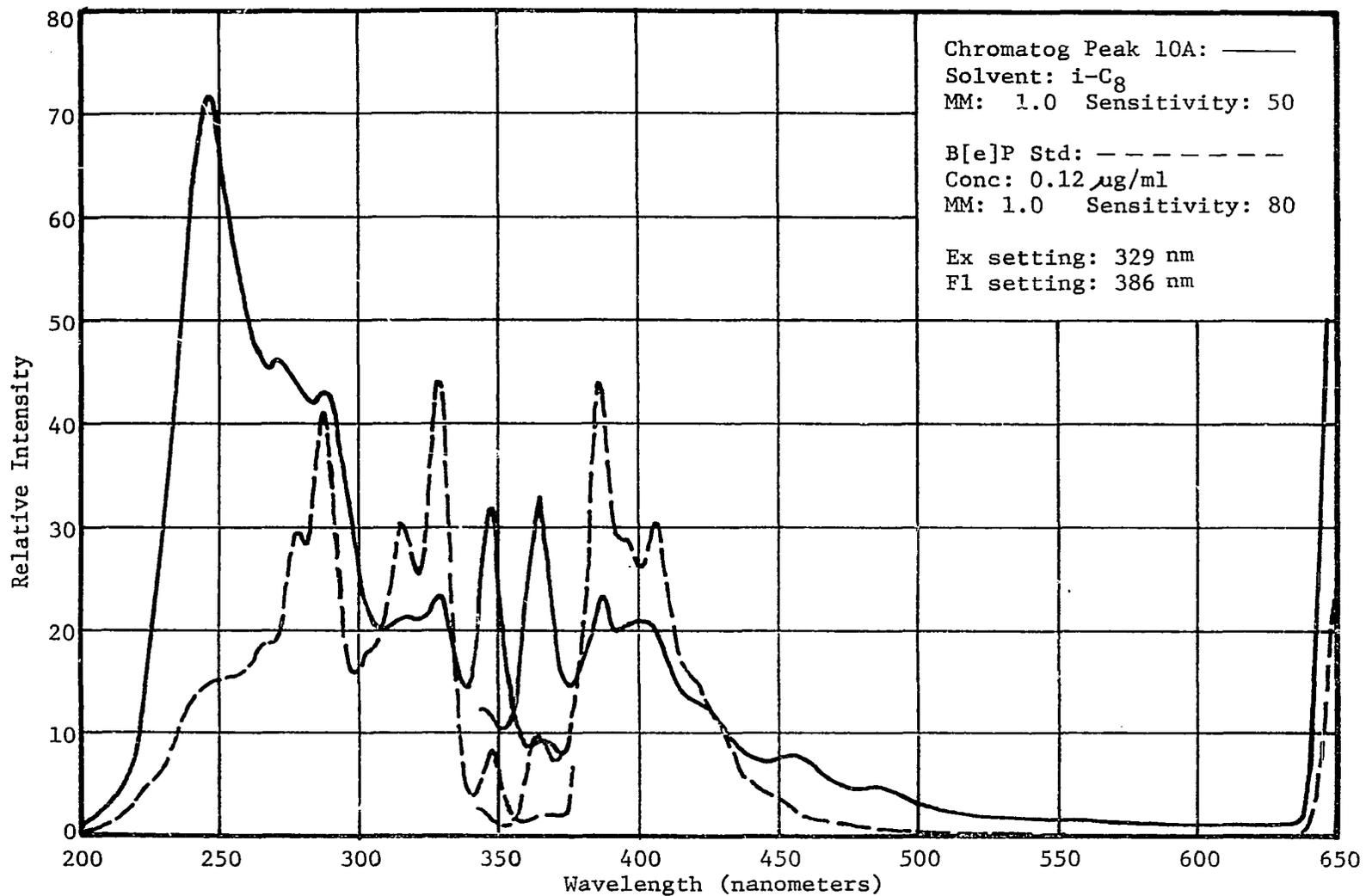
Excitation and fluorescence spectra of chromatographic Peak 7 from carbon black extract and benz[a]anthracene.



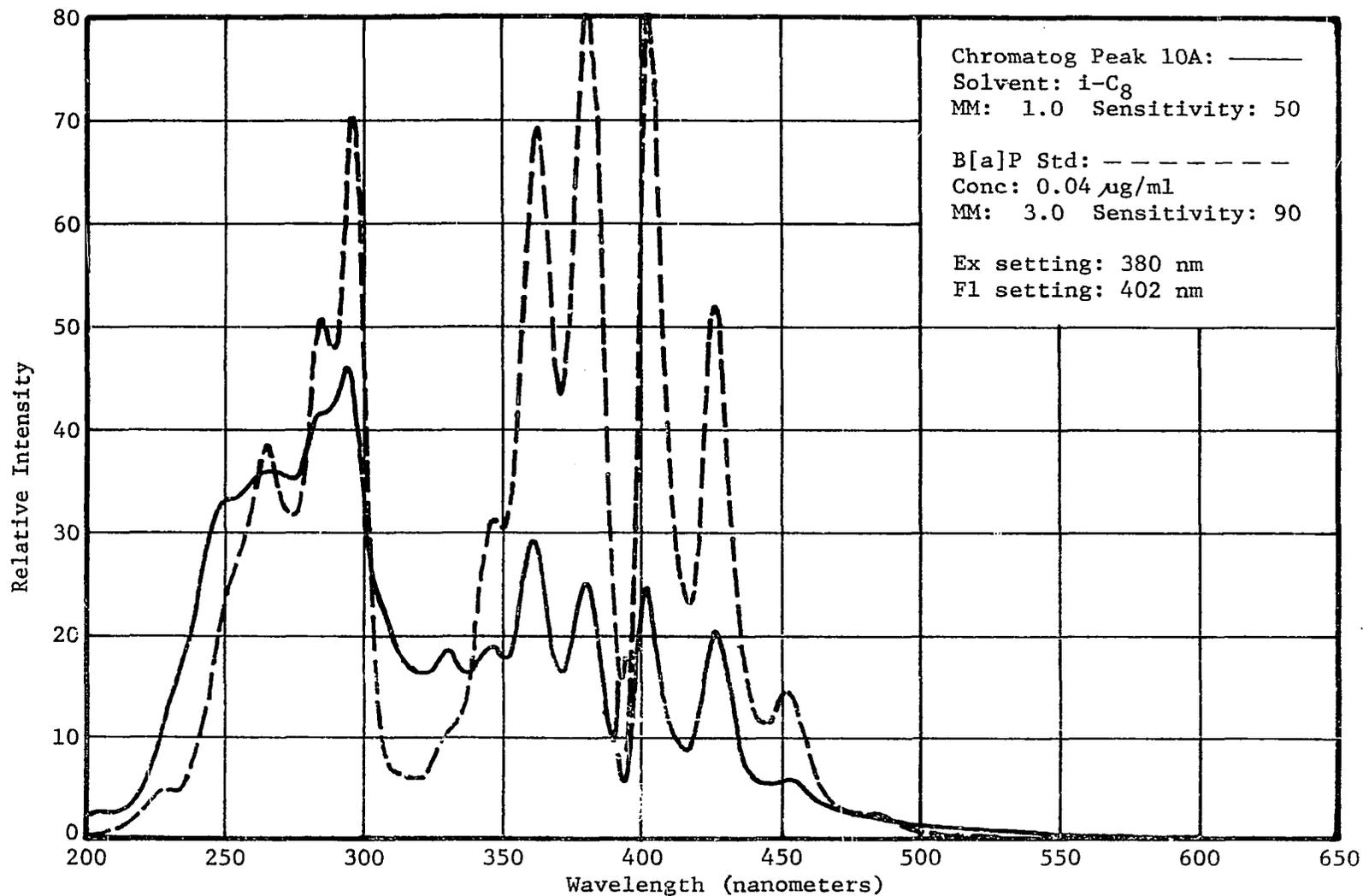
Excitation and fluorescence spectra of chromatographic Peak 7 from carbon black extract and chrysene.



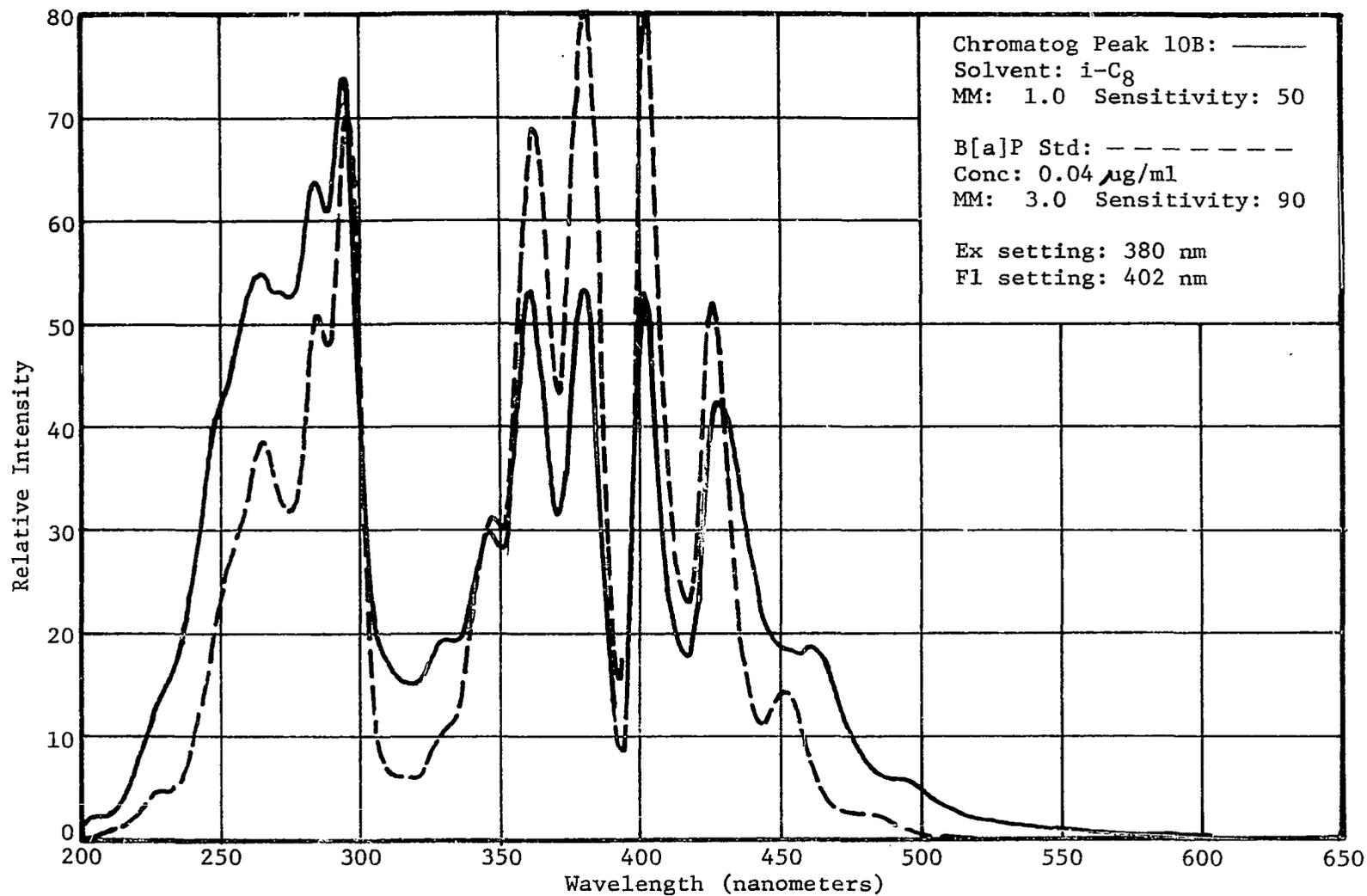
Excitation and fluorescence spectra of chromatographic Peak 9 from carbon black extract.



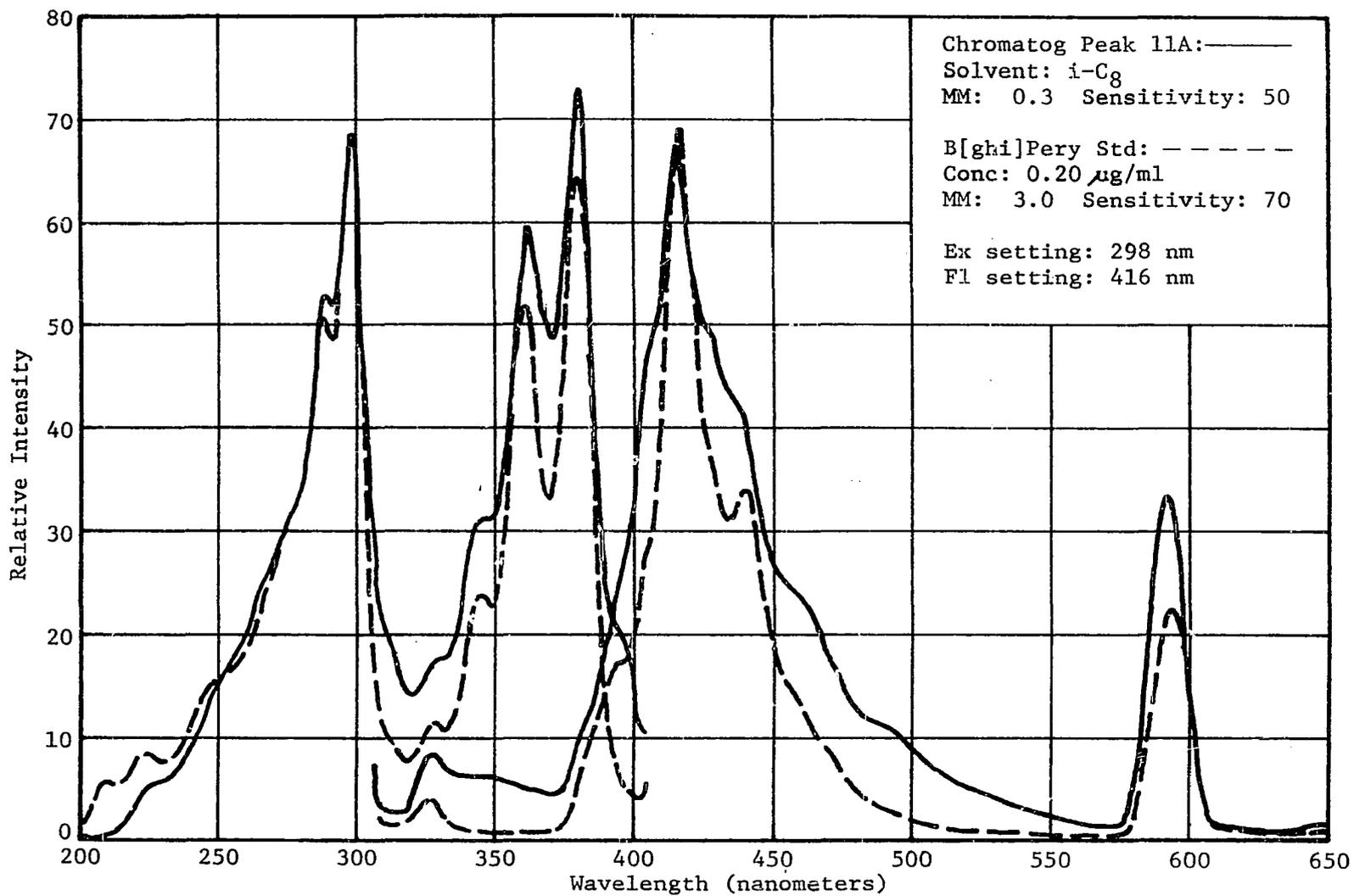
Excitation and fluorescence spectra of chromatographic Peak 10A from carbon black extract and benzo[e]pyrene.



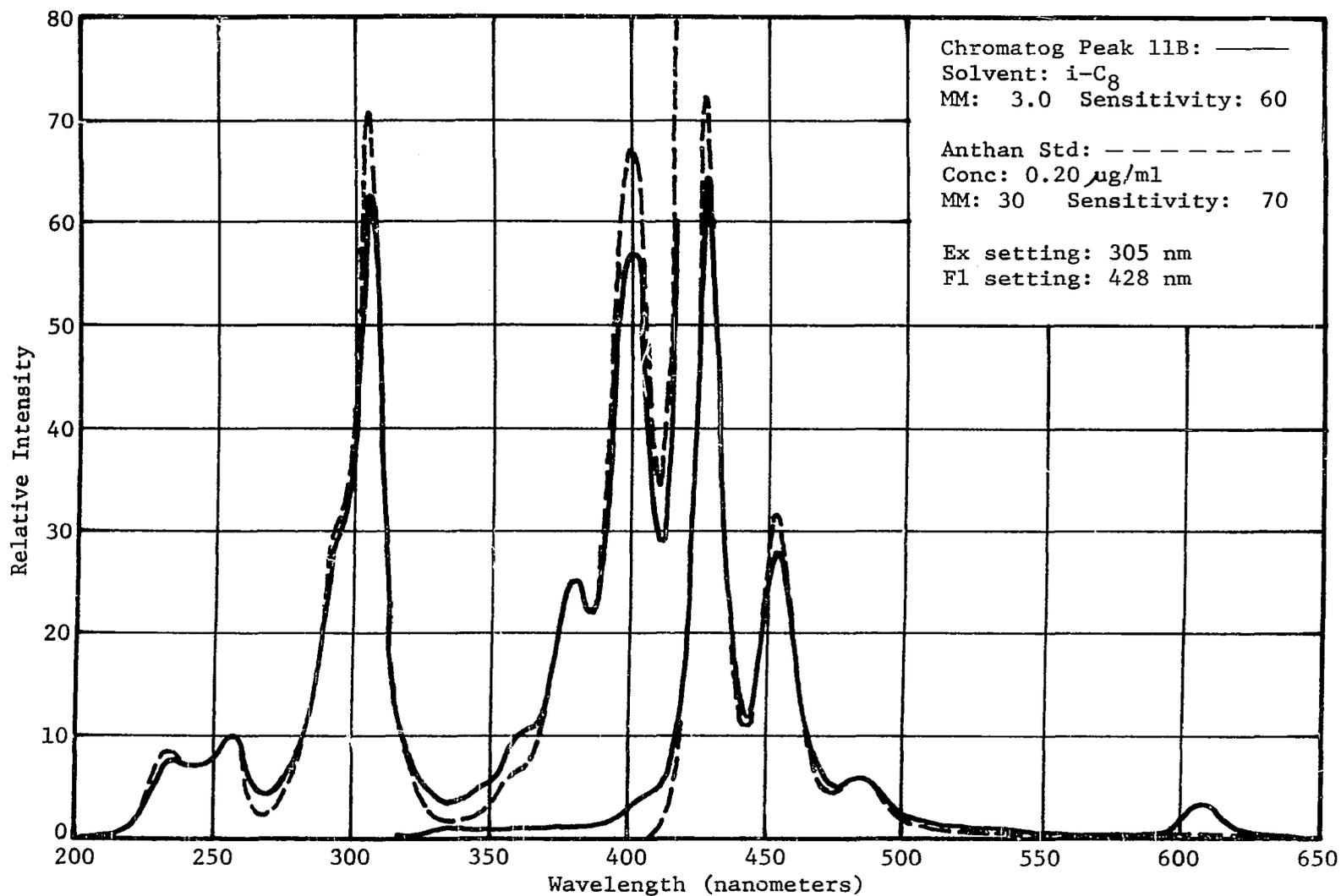
Excitation and fluorescence spectra of chromatographic Peak 10A from carbon black extract and benzo[a]pyrene.



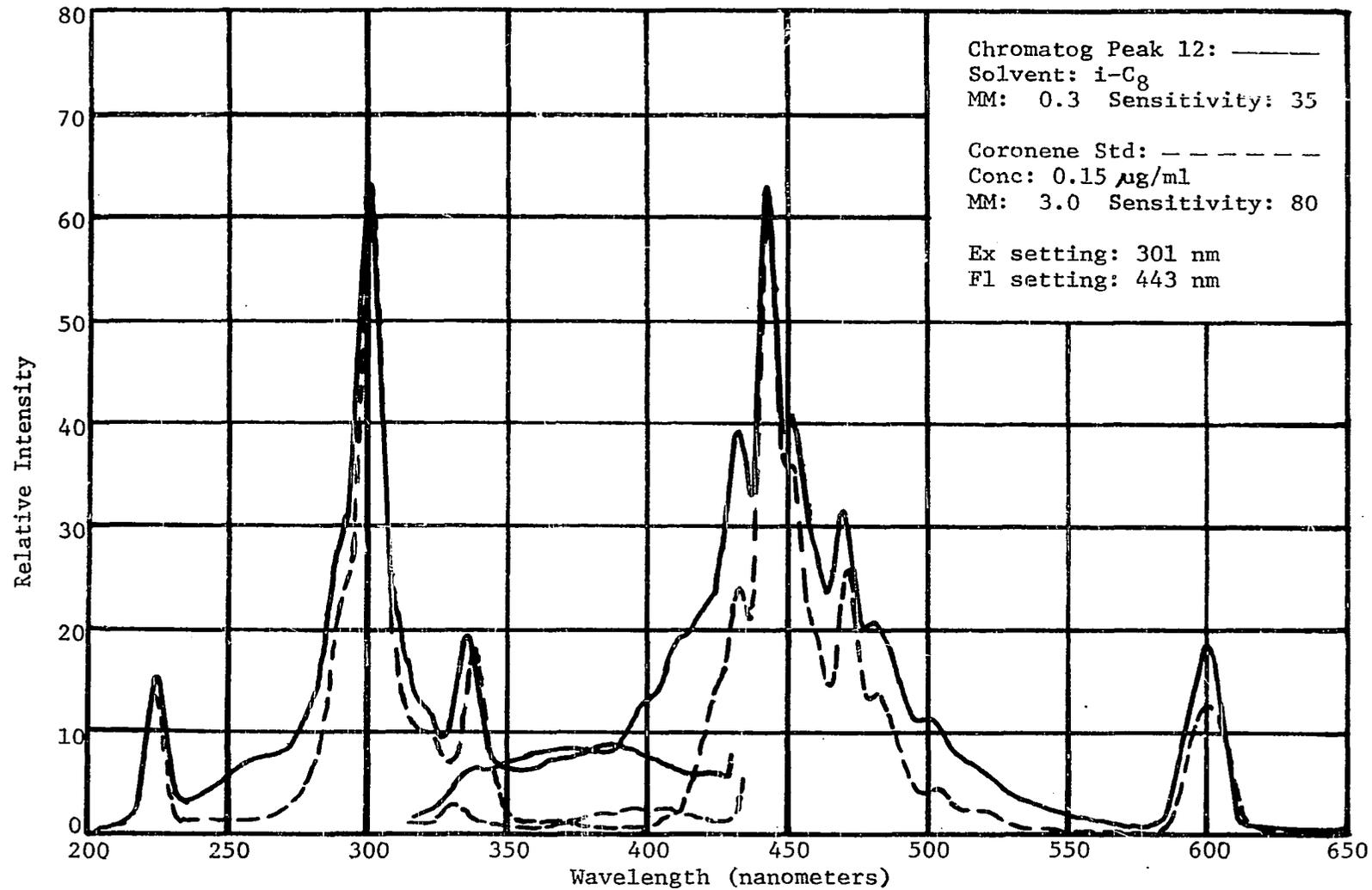
Excitation and fluorescence spectra of chromatographic Peak 10B from carbon black extract and benzo[a]pyrene.



Excitation and fluorescence spectra of chromatographic Peak 11A from carbon black extract and benzo[ghi]perylene.



Excitation and fluorescence spectra of chromatographic Peak 11B from carbon black extract and anthanthrene.



Excitation and fluorescence spectra of chromatographic Peak 12 from carbon black extract and coronene.