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DEVELOPMENT OF A TECHNIQUE FOR QUANTITATIVE SEPARATION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM CARBON BLACK

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DEVELOPMENT OF A TECHNIQUE FOR QUANTITATIVE SEPARATION

OF POLYCYCLIC AROMATIC HYDROCARBONS

FROM CARBON BLACK

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To my Mother

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DEVELOPMENT OF A TECHNIQUE FOR QUANTITATIVE SEPARATION OF POLYCYCLIC AROMATED HYDROCARBONS FROM SRF CARBON BLACK

By Abdul Hamid Qazi

Major Professor: Carl A. Nau, M.D.

This research project was designed to develop a sensitive, accurate, and rapid method for separation, detection, identification, and quantitation of polycyclic aromatic hydrocarbons (PCAHs) in semi-reinforcing furnace carbon black. The method which was finally developed consisted of: a) Soxhlet extraction of the carbon black with benzene, b) gas chromatography of the carbon black extract on a 5 ft x 0.25 in, OD, stainless steel column, c) enhancement of each separated peak by the addition of a suspected PCAH, and d) collection of the peaks separated by gas chromatography and confirmation of their identity by comparison of their excitation and fluorescence spectra with those of known standard PCAHs. The PCAHs identified in carbon black were: anthracene, phenanthrene, fluoranthene, pyrene, benzo(mno)-fluoranthene, chrysene, 1.2-benzanthracene, 9,10-dimethyl-1.2-benzanthracene, benzo(e)pyrene, benzo(a)pyrene, perylene, o-phenylene pyrene, 1.12-benzoperylene, anthanthrene, and coronene.

Polycyclic aromatic hydrocarbons identified above were quantitated by two different procedures: a) by calculating the area of each separated peak and then reading the concentration directly from the plot of peak area against the concentration of the corresponding PCAH standard, and b) by recording the excitation and fluorescence spectra of the unknown PCAHs along with those of the standard PCAHs at known concentration. Comparison of the peak heights (or relative intensity) under similar conditions yielded the concentration of the PCAH. Results were in good agreement.

DEVELOPMENT OF A TECHNIQUE FOR QUANTITATIVE SEPARATION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM CARBON BLACK

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Carcinogenicity, Structure, and Reactivity

Cancer was recognized as an occupational disease as early as 1775, when chimney sweeps were found to suffer from a high incidence of scrotal cancer (1). Pott was the first to report his observations concerning the disease and its prognosis. He states:

> The fate of these people seems singularly hard; in their early infancy, they are most frequently treated with great brutality, and almost starved with cold and hunger; they are thrust up narrow, and sometimes hot chimnies, where they are bruised, burned, and almost suffocated; and when they get to puberty, become peculiary liable to a most noisesome, painful, and fatal disease.
>
> Of this last circumstance there is not the least doubt, though perhaps it may not have been sufficiently attended to, to make it generally known. Other people have cancer of the same parts; and so have others, besides lead-workers, the Poictou colic; and the consequent paralysis; but it is nevertheless a disease to which they are peculiarly liable; and so are chimney sweepers to cancer of the scrotum and testicles.
>
> The disease, in these people, seems to derive its origin from a lodgement of soot in the rugae of the scrotum, and at first not to be a disease of the habit. . . . but here the subjects are young, in general good health, at least at first; the disease

> brought on them by their occupation, and in all probability local; which last circumstance may, I think, be fairly presumed from its always seizing the same parts; all this makes it (at first) a very different case from a cancer which appears in an elderly man, whose fluids are become acrimonious with time, as well as other causes; or from the same kind of complaint in women who have ceased to menstruate (1).

Since then, there has been a continual search for the external causes, prevention, and treatment of cancer. However, the search for such causes had to wait for more than 140 years until the Japanese workers, Yamagiva and Ichikawa (2), succeeded in inducing skin tumors by repeated applications of coal tar to the skin of experimental animals (rabbits). According to Schoental (3), Block and Dreifuss obtained evidence that the carcinogenic factor in coal tar was free from sulfur and nitrogen, formed picrates, and was probably of the aromatic hydrocarbon type.

In 1930, Kennaway and Heiger (4) reported that dibenz(a,h)-anthracene (an aromatic hydrocarbon) was the first pure substance known to produce cancer. Soon after this discovery, Cook, Hewett, and Heiger, in 1933, reported the isolation, identification, and synthesis of the first pure environmental polycyclic aromatic hydrocarbon (PCAH), benzo-(a)pyrene from coal tar (5). Benzo(a)pyrene or 3.4-benzopyrene (abbreviated BAP) was later shown to be a potent carcinogen (6). This discovery led to a rapid awakening of interest in the isolation and identification of PCAHs and other carcinogenic compounds from many products. Henry (7) reviewed the extensive occurrence of cutaneous cancer among workers handling coal tar and its products during the late Nineteenth and early Twentieth Century. He concluded that pitch, tar and tar products, shale oil, mineral oil, and bitumen were responsible for a

high incidence of skin cancer.

In 1939, Cottini and Mazzone (8) demonstrated that BAP when applied to skin for a prolonged period can cause cancer in man. Rhoads et al. (9) confirmed the observation of Cottini and Mazzone. Thousands of recent animal studies have also varified the carcinogencity of BAP (10, 11, 12). Hendricks and co-workers (13), in an epidemiological study, reported a high incidence of scrotal cancer among wax pressmen. In addition to skin tumors, Rigdon et al. (14) and Neal and Rigdon (15) demonstrated the production of gastric carcinoma and gastric tumors in mice fed BAP. Again, Rigdon et al. (16) and Uematsu (17) have separately shown the production of leukemia in mice fed BAP. In fact, BAP has been and is one of the standard carcinogens used in cancer research today. Wallcave and co-workers (18) have recently demonstrated the production of skin tumors in mice by painting their skin with petroleum asphalt and coal-tar pitches of known PCAH content.

Several mechanisms have been proposed in the literature as explanation to the chemical carcinogenesis. The mechanism proposed recently by Heidelberger (19, 20) seems interesting and more convincing. He believes that chemical carcinogens act through a cellular mechanism by transforming or converting normal cells into cancer cells. Mondal and Heidelberger (21) succeeded in transforming individual normal mouse cells into malignant cells with 3-methylcholanthrene and thus provided evidence in support of the above mechanism. Berenblum (22, 23) and Van Duuren (24) showed that this carcinogenic process in skin occurs in two stages known as "initiation" and "promotion". Initiation is an irreversible change specific for carcinogens, which transforms normal cells

into "dormant" tumor cells, a process similar to mutation. Such changed cells may remain dormant for very long periods unless stimulated to activity either by additional treatment with a carcinogen or with a "promoting" agent. The latter can be a non-specific cell irritant, such as croton oil or phorbol ester, iodoacetic acid, phenols, detergents, and even mechanical tissue injury.

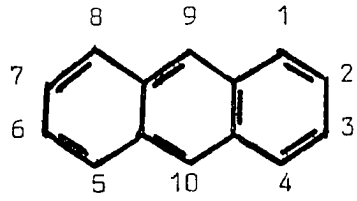
Berenblum (22) described three main types of modifying factors. These modifying factors can cause inhibition (anticarcinogenesis), or augmentation (co-carcinogenesis), or a qualitative change of a particular biological process. He further listed seven ways by which a carcinogen can operate. Gelboin et al. (25) showed that actinomycin D inhibits tumor formation by 9,10-dimethyl-1.2-benzanthracene (DMBA) in mouse skin. Falk et al. (26) found that some PCAHs, such as phenanthrene and anthracene, are able to inhibit the carcinogenicity of BAP. (See Appendix A for abbreviations.)

Polycyclic aromatic hydrocarbons, their derivatives, and analogs form a large group of carcinogenic substances. At present, more than 250 of them are known to possess several unique features which distinguish them from most of the recently discovered carcinogen. They act at the site of application, the effective dose is very small, and they have been found to induce tumors in almost every tissue and animal species on which they have been tested. Carcinogenic activity has been found mainly in certain appropriately substituted tri-, tetra-, penta-, hexa-, and a few higher cyclic aromatic hydrocarbons.

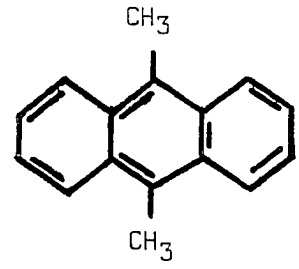
Methyl substitution at certain position(s) of an otherwise inactive aromatic hydrocarbon can change the hydrocarbon to a very potent

carcinogen. For example, anthracene (see Figure 1) has not been shown to possess carcinogenic activity but its dimethyl derivative, 9,10-dimethylanthracene is a weak carcinogen. Similarly, 1.2-benzanthracene is only a weak carcinogen as compared to its dimethyl derivative, 9,10-dimethyl-1.2-benzanthracene, which is a potent carcinogen. However, methyl substitution can also reduce the carcinogenic activity of the parent compound from very potent to almost zero activity. Thus, the introduction of one methyl group into the 5-position of the very potent carcinogens 3.4,8.9- and 3.4,9.10-dibenzpyrenes reduced the activity, and introduction of two methyl groups abolished it completely; the 5,10-dimethyl-3.4,8.9-dibenzpyrene and 5,8-dimethyl-3.4,9.10-dibenzpyrene proved to be inactive (3, 27, 28, 29, 30).

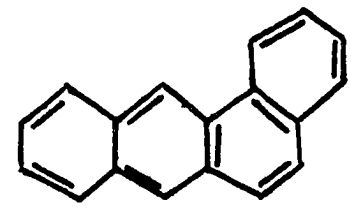
In PCAHs a certain degree of molecular complexity and chemical reactivity is required for carcinogenic action. Theoretical chemists and physicians believed that there should be a way by which carcinogenicity of such molecules can be calculated and predicted from the electronic structure without actually testing them on animals. Badger (31) suggested the presence of phenanthrenoid 9.10-bond as essential for carcinogenic activity. This does not explain the carcinogenicity of 9,10-dimethylanthracene which does not possess the phenanthrenoid 9.10-bond. Schmidt (32) attempted to correlate carcinogenicity with the electronic structure of PCAHs. Coulson (33) next calculated and assigned electron densities to various regions. Pullman and Pullman (34) reviewed and extended Schmidt's electronic theory as it relates to chemical reactivity and carcinogenic activity. They built their model on 1.2-benzanthracene which is a weak carcinogen. Taking 1.2-benzan-



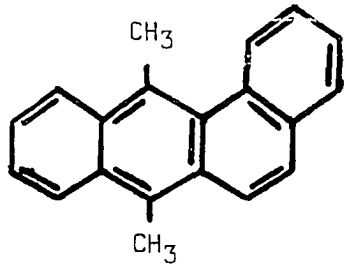
Anthracene



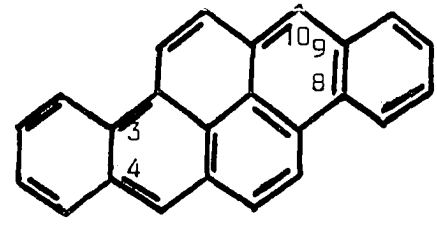
9,10-Dimethyl-anthracene



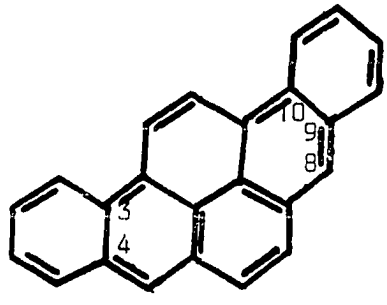
1.2-Benzanthracene



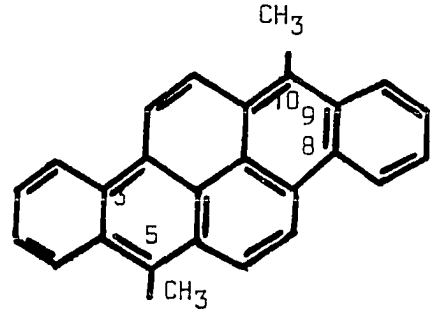
9,10-Dimethyl-1.2-benzanthracene



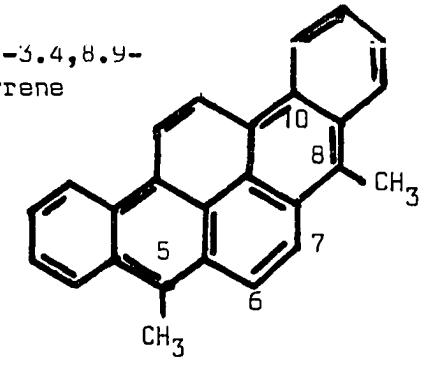
3.4,8.9-Dibenzpyrene



3.4,9.10-Dibenzpyrene



5,10-Dimethyl-3.4,8.9-dibenzpyrene



5,8-Dimethyl-3.4,9.10-dibenzpyrene

Figure 1.--Structure and name of polycyclic aromatic hydrocarbons and their methyl-derivatives.

thracene as a model compound, they explained their theory by assigning three active centers (or regions) according to high electron densities as shown in Figure 2. These active centers are:

K-region - (9.10-phenanthrenoid bond) attaches with the cellular component and is primarily responsible for carcinogenic activity;

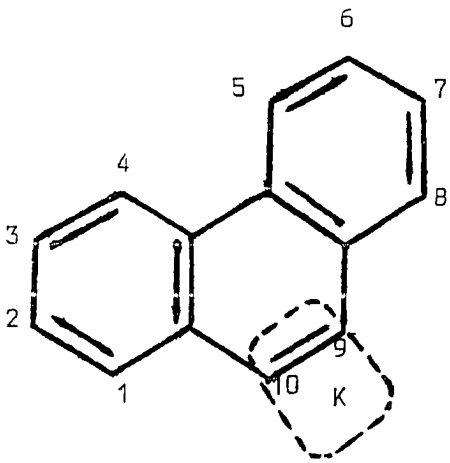
L-region - (anthracenoid meso-carbons) where addition and substitution occur; L-region remains inactive for K-region to be carcinogenic; and

M-region - susceptible to metabolic oxidation and hydroxylation.

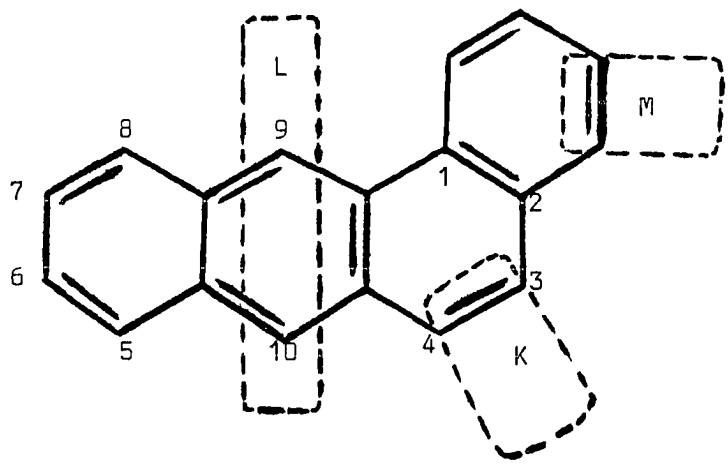
With a few exceptions, Pullman and Pullman (34) successfully predicted carcinogenic activity in a number of closely related series of carcinogenic compounds. These exceptions are: 3.4-benzophenanthrene, 1.2-benzochrysene, 5.6-benzochrysene, and anthanthrene, which were all predicted to be active while, in fact, they are inactive (27, 28, 29). Also, the theory fails to explain the effect of methyl substitutions in the angular ring of 1.2-benzanthracene or the increase in potency observed in the 9- or 10-position of 1.2-benzanthracene (28).

Sources of PCAHs in Our Environment

One of the major sources of PCAHs is from the incomplete combustion of fossil fuels such as coal, petroleum, natural gas, or more generally, compounds containing carbon and hydrogen (27, 28, 35, 36, 37). These sources can be divided into natural sources of PCAHs and man-made or technologic sources. Natural sources of PCAHs include uncontrolled combustion, such as that in forest fires and decaying organic matter. Emission data on natural sources are not available and are generally considered as negligible. Engdahl (35) and Altshuller (36)



Phenanthrene



1.2-Benzanthracene

Figure 2.--Numbering and region designation of phenanthrene and 1.2-benzanthracene.

listed many sources of PCAHs in the air. Man-made sources can be further divided into stationary sources and mobile sources. About 90 per cent of the emissions come from stationary sources and the remaining 10 per cent from mobile sources (36). Van Lehmden et al. (38) reported on the PCAH emissions from several selected industrial processes. Hangebrauk and co-workers (39), Muhich et al. (40), and others (41, 42) reported on the PCAH emissions from several different sources. Table 1 gives the estimated BAP emissions from heat and power generating sources (using coal, oil, gas, and wood) in the United States. Hangebrauk et al. (39) concluded that the most important source of BAP of these four was the inefficient combustion of coal in hand-fired residential furnaces. This is quite obvious from Table 1.

The intentional combustion of solid wastes as a method of disposal, as well as accidental uncontrollable combustion processes, can contribute significantly to the overall PCAH emissions. Hangebrauk et al. (38) reported the BAP emission factor for municipal and commercial incineration of wastes such as those collected from households, business, restaurants, municipal, and agricultural refuse and junked automobiles as well as from catalytic cracking of petroleum. Muhich et al. (40) and others (37, 41, 42, 43) reported BAP emission data from enclosed incineration of community solid waste, and open burning of municipal, domestic, industrial, and forest and agricultural wastes as well as vehicle disposal and coal refuse fires. All these data are summarized and are presented in Table 2 as total estimated BAP emmissions in tons per year from all stationary sources in the United States. It is clear from the table that solid waste disposal contributes

TABLE 1

ESTIMATED BAP EMISSION FROM HEAT AND POWER GENERATION SOURCES IN UNITED STATES[a]

Type of Unit	Gross Heat BTU/Hour x 10^6	BAP Emission[b] Factor ug/10^6 BTU	BAP Emission Tons/Year
Coal:			
Hand-Stoked Residential Furnaces	0.1	1,700,000-3,300,000	420
Intermediate Units (Chain-Grate and Spreader Stokers)	60-250	15-40	10
Coal Fired Steam Powered Plants	1,000-2,000	20-400	1
Oil:			
Low-Pressure Air-Atomized	0.7	900	2
Other	0.02-21	100	
Gas:			
Premix Burners	0.01-9	20-200	2
Wood:	-	50,000	40

[a]Data from (36, 39, 40, 41, 42)

[b]BAP emission factor is an index used for total PCAH emission

TABLE 2

TOTAL ESTIMATED BAP EMISSION FROM ALL STATIONARY SOURCES IN THE UNITED STATES

Sources of BAP	BAP Emission Tons/Year	Total	Per Cent Total
Heat and Power Generation:			
Coal	431		
Oil	2		
Gas	2		
Wood	40	475	38
Refuse Burning:			
Enclosed Incineration	34		
Open Burning	214		
Coal Refuse Fires	340	588	46
Industrial Activities:			
Petroleum Catalytic Cracking	6		
Asphalt Air-blowing	1		
Coke Production	200	206	16
Grand Total	1,269 Tons/Year		

the highest amount of BAP followed by the heat and power generating sources and other industrial activities. Solid waste disposal is an increasing problem in the United States and its potential for even higher tonnage of BAP per year can be predicted in the future.

Another 10 per cent of the total PCAHs emission comes from mobile sources. A significant source of atmospheric PCAH is the conventional automobile powered by an internal combustion engine. Shabad (44), in a review paper, has shown that a Volga car exhausts 160 microgram (ug) of BAP during a 3-hour period on one of the main Moscow highways. He also found that one diesel engine, in starting and stopping 100 times, discharged about 500 ug of BAP. In soil samples collected near the sidewalk of a Moscow street (one-way traffic, about 500 cars per hour), he found 21,150 ug of BAP per kilogram (Kg) of soil while the mean background pollution of Moscow soil is about 500 ug per Kg of soil. The greatest amount of BAP is produced during the deceleration cycle, that is, near traffic lights, in places of traffic jams, etc. Hangebrauk et al. (39, 45) reported BAP emission from gasoline-powered trucks and automobiles. His data show a wide variation in emission factors, from 70 to 1,500 ug/gallon. Table 3 gives an estimate of vehicular BAP contribution. From nationwide fuel consumption data, Hangebrauk et al. (39, 45) concluded that the BAP emission is due to incomplete combustion. The efficiency of combustion depends largely on air to fuel ratio, control devices, maintenance, etc. Similar studies on diesel-powered trucks and buses, and gasoline-powered two-cycle engines are not available.

In 1972, Shabad and Smirnov (46) conducted a remarkable study

TABLE 3

ESTIMATED BAP EMISSION FROM GASOLINE POWERED ENGINES IN THE UNITED STATES

Vehicle Type	Fuel Consumed gal/Year	BAP Emission Factor ug/gal	BAP Emission Tons/Year
Gasoline Powered:			
Automobile	56.4×10^9	170	10
Trucks	24.2×10^9	500	12
Total	-	670	22

at a Moscow airport and estimated the BAP contents of the soot and exhaust products of aviation engines, both piston and turbine. Their results are summarized in Table 4.

TABLE 4

BAP EMISSION FROM MODERN AIRCRAFT ENGINES IN THE U.S.S.R.

Engine Type	BAP Release			Range
	Soot ug/Kg	Test Stand ug/Kg	Exhaust mg/min	mg/min
TU-104 Turbo Jet	350 N[a]	30,000 SC[c]	2-4 at 10,500 rpm 10 at Max speed	2-10
IL-14 Piston) An-2 Piston)	250 EP[b]	27,000 SC	4 at 8,000 rpm 8 at Max speed	2-8

[a]N - nozzles of turbo jet
[b]EP - exhaust pipes of piston
[c]SC - soot collectors

They included in this study, the distribution of BAP in the vegetation and soil around the runway as well as in the runway sweepings. Their findings were:

a) BAP in the runway sweepings amount to 182 ug/Kg;

b) BAP content of vegetation varies from 21.3-5.4 ug/KG for plants and from 7.0-3.1 ug/Kg for the roots of these plants;

c) BAP decreases from 64.3 to 15.5 ug/Kg soil as the collection point moves away from the taxiway, which is the center of the carcinogenic discharge; BAP content along the runway varies from 14.0 ug/Kg at the middle to 64.3 ug/Kg at the ends (take off point) of the runway; and BAP content of the soil decreases with the distance from the end of the runway (68.0 ug/Kg at the end to 1.3 ug/Kg at 1.5 kilometers); and

d) Control soil samples in the rural districts vary from 0.3 to 0.8 ug/Kg soil.

They further concluded that a modern aircraft releases into the atmosphere from 2 to 10 mg of BAP per minute. Extracts of aviation engine soot when applied to mice skin induced malignant tumors. The ground within the airport was polluted with BAP and its content diminishes with the distance from the runway. The concentration of carcinogenic hydrocarbon in aircraft exhausts is dependent on the working regime of the engine and the character of fuel combustion (46).

It is very unfortunate that similar studies on PCAH emission from aircraft have not been done in the United States, and quantitative data of total PCAH pollution load from this very important source in the United States are not available. However, Lozano et al. (47) and Poth and Lozano (48) gave evidence that the exhaust from jet aircraft engines contributes to the air pollution in total hydrocarbons, aromatics, and particulates. The Environmental Protection Agency (49) has also reported some data on total hydrocarbon and particulate matter from aircraft

emissions. By comparing the amount of BAP in the exhaust gases of these jet-engines with that of average urban air, and with the number and frequency of jet airplanes flying today, the significance of this source is quite obvious. It is not only one of the major sources of air pollution but also a universal distributor of carcinogens in the atmosphere, soil, and vegetation.

Moore and Katz (50) reported on the particulates of diesel exhausts in railway tunnels and identified 9 PCAHs consisting of: fluorene, anthracene, pyrene, fluoranthene (F), 1.2-benzanthracene, 1.2-benzopyrene (BEP), BAP, 1.12-benzperylene, and coronene. Falk et al. (51) isolated 8 PCAHs, including BAP, from the benzene extracts of rubber stoppers and automobile tires. Thompson and co-workers (52) and Thompson (53) identified vehicle tire rubber in roadway dusts. Smith et al. (54) separated and identified 5 PCAHs, such as BAP, BEP, F, pyrene, and coronene, from rubber dust. These authors believed that tire rubber and dust from bitumen road surfaces are significant sources of PCAHs as air pollutants. Kurker (55) believed that in addition to wear and tear of tires on the millions of cars, trucks, buses, etc. on our highways, the disposal of rubber tires in the United States is a nationwide problem. Further, Cole et al. (56), in an interesting epidemiological study in eastern Massachusetts, found a good correlation between bladder cancer and occupations in such materials as dystuffs, rubber, leather and leather products, paint, and organic chemicals. Among these occupations, relative risks of bladder cancer in men who were ever employed in the rubber industry and in the leather industry were considered as statistically significant. Also these five occupations account for 7.3

cases of lower urinary tract cancer for 100,000 men between the ages of 20 and 89.

In the last half century, the incidence of lung cancer among the male population has been rising alarmingly in many countries, particularly in Britain and the United States. Kennaway and Lindsey (57) reported cigarette smoking and air pollution as etiological factors. The search for possible carcinogenic constituents in tobacco smoke revealed the presence of several carcinogenic polycyclic hydrocarbons and other compounds listed below (57, 58, 59):

a) Arsenious oxide

b) Chrysene

c) 1.2-benzanthracene

d) 6.7-cyclopenteno-1.2-benzanthracene

e) 5.6-cyclopenteno-1.2-benzanthracene

f) 1.2,5.6-dibenzanthracene

g) 3-methylpyrene

h) 3.4-benzopyrene

i) 1.2,3.4-dibenzopyrene

j) 3.4,9.10-dibenzopyrene

k) 1.12-benzoperylene

l) 3.4-benzofluoranthene

m) 10.11-benzofluoranthene

n) 1.2,5.6-dibenzacridene

o) 1.2,7.8-dibenzacridene

p) 3.4,5.6-dibenzcarbazole

However, the quantities of these are very small in cigarette smoke.

Shabad (60), in an experimental study on the link between air pollution and lung cancer, concluded that BAP was present in both the atmosphere and in Russian cigarettes. The tarry products from air pollution and cigarette smoke produced malignant tumors in mice by skin painting and subcutaneous injection. Stocks (61), in an epidemiological study, reported a good correlation of cigarette smoking and air pollution to lung cancer mortality. He reported the average concentrations of total smoke, BAP, 1.12-benzoperylene, pyrene, fluoranthene, and sulfur dioxide in seven locations for a period of 1 to 3 years. He also found a significantly higher lung cancer rate in four locations (Dublin, Liverpool, Belfast, and North Wales) where the average smoke concentration was higher (174 mg/1000 cubic centimeters) as compared to areas of lower average smoke (56 mg/1000 cubic meters).

According to this survey, the major sources of atmospheric polycyclic aromatic hydrocarbon pollutants are summarized as:

a) Mobile sources such as transportation which include the following:

   (1) exhausts from automobiles, trucks, buses, diesel locomotives, etc.;

   (2) emissions from jet aircraft engines;

   (3) dusts from rubber tire and asphalt disintegration;

b) Stationary sources such as:

   (1) heat and power generation;

   (2) refuse burning;

   (3) industrial processes

c) Others such as:

(1) smoke and tar from cigarettes (personal air pollution);

(2) natural sources such as forest fires and spontaneous combustion.

Carbon Black

Carbon black is an amorphous form of finely divided carbon produced industrially by incomplete combustion or thermal decomposition of natural gas, oil, or both. It is primarily (about 90 per cent) used in reinforcing rubber and as a pigment in the ink and paint industry. Carbon black has been used as a food additive and in cosmetics. Nau et al. (62), in their studies of carbon black ingestion, pointed out the extensive use of carbon black as a coloring agent for certain foods (jelly beans, chocolate, candies, gum drops, licorice) and in inks and rubber goods used in food processing (conveyor belts, seals, gaskets, tubings, etc.).

Commercial carbon blacks are produced by a number of methods. Four principal methods described by Nau et al. (62) are:

a) Channel blacks are produced by the impingement of natural gas flames upon slowly moving channel irons.

b) Lampblacks are produced by burning liquid fuels (petroleum oil, tars, and aromatic residues) in specially designed pans.

c) Furnace blacks are prepared by the partial combustion of gas, oils, or gaseous hydrocarbons.

d) Thermal blacks are produced by thermal decomposition of hydrocarbon in preheated furnaces.

The market demand for various carbon blacks depends upon their physical and chemical properties such as particle size, anisotropy, and surface characteristics. These properties also distinguish them from each

other. Carbon black particles are essentially spherical in shape.

Historically, the channel blacks were first commercially produced from natural gas in New Cumberland, West Virginia in 1872 (62). They are now produced in several other states, Texas, Louisiana, New Mexico, Arkansas, Oklahoma, and Kansas. Until 1940, channel blacks accounted for almost 80 per cent of the domestic production of carbon black. Due to rising costs and higher demand for natural gas, the production of channel blacks is reduced to less than 20 per cent. They are among the most expensive carbon blacks produced today. However, the channel blacks have the smallest particle size varying from 50 to 350 $\mathring{A}$ in diameter, have less than 0.1 per cent ash content, and have very little benzene extractable material. The rubber reinforcing channel blacks are primarily in the 250-350 $\mathring{A}$ diameter particle size range (62). The size of the carbon particle has its importance in the rubber industry, since the strength and hardness of rubber depends on the size of the carbon particle. The smaller the particle size, the more strength and hardness carbon gives the rubber. About 30 to 50 per cent by weight of carbon black is used in rubber for tires. It not only reinforces the rubber tire, but also increases the length of time of the tread wear about 10 times.

Furnace blacks are largely used in the manufacture of tire rubber and have a particle size which may vary from 500 to 800 $\mathring{A}$ in diameter. The ash content of carbon black consists primarily of the oxides of iron, silicon, and magnesium and is believed to arise from the hard water used for quenching. The furnace blacks have an ash content of about 0.1 to 1.0 per cent. The benzene extractable material for furnace

blacks was reported to be 0.1 to 0.3 per cent (62, 63).

Thermal blacks, on the other hand, are the coarsest carbon blacks with a very large particle size ranging from 1000 to 5000 Å in diameter. Large size particles have the advantage in that more black can be loaded into the rubber stock, thus lowering the price of the product by diluting out the valuable rubber latex. Thermal blacks have more than 1 per cent benzene extractable material. They are used mainly as dilutents and have very little effect on any of the physical properties of the rubber (64). Carbon black is essentially carbon combined with residual hydrogen and chemically combined oxygen on its surface. Nau et al. (62) state:

> The oxygen is chemisorbed on the carbon 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.

The nomenclature, general properties, and various uses of carbon blacks are also discussed in this paper (62). Carbon black can be thought of as an agglomerate of pericondensed polynuclear hydrocarbons (62). Thomas et al. (65) state:

> X-ray diffraction has definitely established that soot particles exhibit the hexagonal symmetry of graphite like crystals. Numerous well developed graphitic platelets, having a carbon skeleton analogous to a large polynuclear aromatic molecule consisting of 50 to 100 pericondensed rings, comprise the layer planes in a soot particle. The platelets in adjoining planes are stacked roughly parallel to one another but random in orientation. Several loosely bound adjacent platelets comprise a cyrstallite, and several thousand interconnected crystallites constitute the individual soot particle.

They also indicate that small channel blacks have a porous structure

while furnace and thermal blacks do not. Their studies have further shown that PCAHs are attached to the surface of the particles by adsorption only.

In the United States as well as in some European countries, the carbon black production has increased by several fold since World War II (62, 66). In 1969, about 2.8 billion pounds were used by the rubber industries in the United States alone. The newer use of carbon black is in the plastic industry.

In early studies, soot was found to cause skin cancer. Sir Percival Pott, in 1775, reported the facial and scrotal cancer among chimney sweeps. However, there has been some controversy about the carcinogenicity of the soot and carbon black and their being a potential occupational cancer hazard in industry. Hueper and Payne (67) demonstrated that soot from coffee roasting plants contained BAP and exposure of mice, rats, and guinea pigs to this soot caused precancerous lesions and benign and malignant neoplasms of the skin, connective tissue, stomach, and bladder. They concluded that the soot from coffee roasting plants plays a contributory role in the high incidence, prevalence, and mortality rates in New Orleans.

Passey (68), in 1920, demonstrated experimental skin cancer with extracts of soot which were free of acidic and phenolic compounds. Falk et al. (51) reported that benzene extracts of rubber stoppers and of automobile tires are carcinogenic to mice skin. They also identified eight PCAHs in the benzene extracts, namely, BAP, chrysene, pyrene, alkyl cyclopentenophenanthrene, BEP, 1.12-benzoperylene, alkyl pyrene, and alkyl 1.2-benzanthracene. Falk and Steiner (69) analyzed benzene

extracts of 24 samples of commercial carbon black which included seven channel blacks. They found that no PCAH could be eluted from the channel blacks. In most of the furnace blacks, they identified seven PCAHs: pyrene, BAP, BEP, anthanthrene, 1.12-benzperylene, F, and coronene. They also found some correlation between amounts of benzene extractable PCAHs and average particle size of the carbon blacks. They have also proved that the source of PCAHs in the synthetic rubber is carbon black.

Nau et al. (62, 63) studied the physiological effects of prolonged exposure to carbon black in mice, rabbits, and monkeys. They observed no incidence of carcinogenic effects in these animals when exposure was by ingestion or by direct skin contact. They also noted that carbon black particles had an adsorbed component which when extracted and applied to the skin of mice, produced skin cancer. The component in the adsorbed state was ineffective as a carcinogen. In a later study (70), Nau and co-workers observed that subcutaneous or intraperitoneal injection of carbon black in mice produced no ill effects. However, benzene extracts of furnace blacks, when injected in oil, lead to tumor formation. They also found that neither blood plasma nor gastric juice eluted any significant amount of adsorbed component when channel black was suspended in either medium for up to seven days. In a subsequent study, Nau and Stembridge (71) reported results of carbon black inhalation studies. Hamsters, mice, guinea pigs, rabbits, and monkeys were exposed by inhalation to high concentrations of channel or furnace black for prolonged periods of time. No malignancies were observed even though gross and microscopic changes in the lungs of mice and monkeys were observed.

Falk and Steiner (69) concluded that soot is carcinogenic to skin but possibly not to the respiratory tract epithelium. They explained this difference in behavior by the presence of a lipid solvent (the sebaceous secretions) in the skin and the absence of such solvents in the respiratory tract. Falk et al. (26) state that:

> The liberation of benzopyrene from rubber tire dust by an aqueous protein solution, as is encountered in the lungs occurs rapidly. In the case of soots, particle size plays an important role in determining whether elution of PCAHs will take place.

They also stated that plasma proteins elute BAP from carbon blacks larger than 100 millimicrons in diameter. The smaller particles retain their hydrocarbons and may also adsorb additional hydrocarbons that have been eluted from larger size particles.

The difference between these findings and those of Nau and co-workers (62, 63, 70, 71) could be due to:

a) differences in experimental conditions;

b) different carbon blacks having different properties and composition;

c) different analytical methods; or

d) a combination of the above.

In addition to these differences, the duration of exposure of animals to carbon black in the experiments of Nau and co-workers may be insufficient. It is known that the latent period of occupational cancers in man varies from 20 to 50 years (28, 37). Also, particle size distribution will play a very significant role. A carbon black sample having a large amount of smaller size particles (less than 100 $\mathring{A}$) most probably will not produce any tumor because smaller particles, in addition to retaining their own PCAHs, will also adsorb any eluted PCAH from larger size

particles.

Todd (66) reported the isolation and identification of several PCAHs from "furnace thermal" black. These are: anthracene, F, pyrene, 1.2-benzanthracene, chrysene, BEP, BAP, 1.12-benzoperylene, anthanthrene, and coronene. Recently, Neal and Trieff (72) have reported the isolation of an additional carcinogenic and polycyclic hydrocarbon from carbon blacks.

Analytical Procedures

The PCAHs are essentially odorless solids at room temperature. Although many of them are colorless, some have color. They generally have high melting and boiling points. All known carcinogenic PCAHs absorb light at wavelengths of 350 to 450 nm. In the presence of oxygen the PCAHs may be oxidized. They are relatively more soluble in organic solvents.

Extraction and separation

In general, analytical methods used in the study of carcinogenic PCAHs include extraction, separation, detection, identification, and quantitation. Falk and Steiner (69) extracted PCAHs from carbon blacks by refluxing a known sample of carbon black in benzene for 30 minutes on a hot water bath. When cool the mixture was filtered and the residue was re-extracted. The combined filtrates were evaporated to dryness and the residue was then dissolved in redistilled petroleum ether and chromatographed on a column of activated alumina. This method is tedious and very time consuming and is therefore replaced by Soxhlet extraction, which is a more efficient and continuous method. Extraction

of PCAHs from a weighed sample of carbon black or other sample has been carried out with a suitable solvent (such as acetone, benzene, cyclohexane, etc.) in a Soxhlet extractor for a set period of time, usually 4 to 24 hours (38, 51, 55, 64, 66, 73, 74). The extract containing PCAHs was then made to a known volume with the solvent. The separation of PCAHs were performed on an aliquot portion of this stock solution.

In the past, several techniques have been used for the separation of PCAHs. A broad class separation of the aliphatic and lower molecular weight aromatic hydrocarbons from the higher molecular weight PCAHs has been successfully done by column chromatography in short columns packed with a suitable adsorbent (50, 51, 64, 69, 72, 73, 75, 76, 77, 78, 79). Various adsorbents such as silica gel, Florisil, cellulose acetate, and activated alumina have been used by these workers. Activated alumina seems to be the one most extensively used. Long as well as short columns for complete separation of PCAHs have been used by a few workers with some success (18, 26, 50, 80, 81, 82).

Paper chromatography has been used for further separation of PCAHs (18, 75, 76, 77, 84, 85). It is less expensive but it is not very suitable for routine analysis since it is rather time consuming. Also, it does not separate all the PCAHs. Recently some workers (54, 76, 77, 78, 85, 86, 87) have used thin-layer chromatography (TLC) for further separation of PCAHs. Sawicki and associates (86, 87, 89, 90) have skillfully applied this technique to air pollution research. White and Howard (85) have compared the R_f values for about 30 PCAHs on cellulose and cellulose acetate thin-layer plates. Some researchers prefer TLC because of its neatness, rapidity, and easy handling (no bulky chroma-

tography tanks, etc.). However, TLC does not separate all the PCAHs completely. In order to achieve good separation, Howard et al. (76, 77) have used all three techniques together and in the order- column-, paper-, and thin-layer chromatography. More recently, acetylated paper chromatography seems to be replacing TLC, probably because of better resolution and easy handling. Dubois and co-workers (75) have used acetylated paper chromatography for PCAHs separation and have listed R_f values for 15 PCAHs. (See Appendix B for definition of R_f.)

Sawicki et al. (91) compared eleven methods for the analysis of BAP in air and in other complicated mixtures. They compared factors such as accuracy and precision, man-hours of work, and total analysis time. They have also made some recommendations as to which method should be used under a given circumstance. Most of these methods of analysis for PCAHs were tedious and time consuming. They also suggested further study of gas-liquid chromatography towards improving the separability and analysis of the family of polynuclear aromatic hydrocarbons.

Gas-liquid chromatography (GLC) is one of the most widely used analytical techniques in chemical analysis, particularly in the analysis of compounds of biological importance. It combines simplicity of operation with high speed and sensitivity. Successful GLC analysis of PCAHs is a highly desired goal because of its potential convenience, speed, and reproducibility (92, 93). In addition, GLC has a potential of separating mixtures of compounds that cannot be resolved by the other procedures (such as TLC, column, and paper chromatography, etc.). In a GLC, the separation of the components is achieved on the basis of the different partition coefficients between a stationary phase and a mobile phase.

The stationary phase is a liquid normally having a very high boiling point. The mobile phase is an inert gas generally referred to as the carrier (such as nitrogen, argon, helium).

Significant application of GLC to the analysis of PCAHs dates from 1965, with noteworthy contributions from Wilmshurst (92), DeMaio and Corn (93), and others (52, 66, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104). Wilmshurst (92) discussed a number of previous references that were not suited to low level analysis as is encountered in air pollution studies. He used the flame-ionization (FI) detector and gave retention data for a number of PCAHs on the stationary phases SE-30, SE-52, and QF-1. He further investigated the use of packed and capillary columns for the analysis of PCAHs. Carugno and Rossi (94) noted that a capillary column was capable of separating a large number of PCAHs from a complex mixture, and an electron capture (EC) detector gave a better response for some compounds such as 1,2-benzopyrene and 3,4-benzopyrene. Using SE-52 columns and FI and EC detectors coupled with isothermal and temperature programming, Carugno and Rossi (94) identified many PCAHs from cigarette smoke condensate by comparison of retention indices at linear programmed temperatures of 100°C to 300° C. Chakraborty and Long (95) analyzed soot for PCAHs by programmed temperature GLC, using a dual, 12' x 0.25", SE-52 column and FI detector. The peaks in the chromatograms were identified by comparing the relative retention times with those of pure hydrocarbon standards and by ultraviolet (UV) absorption spectrophotometry of the collected fractions separated by GLC. They collected the fractions by bubbling the effluent helium carrier gas through hexane in a test tube after it had passed

through the exit port of the thermal conductivity detector. For quantitative and better separations, they used dual, 50' x 0.125", SE-52 Hi-Pak columns. However, even with these columns they failed to resolve the components of every peak.

Reid and Halpin (96) described the preparation and use of a simple charcoal tube for the collection of air samples, which were then desorbed in carbon disulfide and analyzed on GLC for halogenated hydrocarbons and PCAHs. Bowman and Beroza (97) reported a more recent and sophisticated use of GLC. They combined the high separative powers of the GLC with the high sensitivity and selective response of the spectrophotofluorometer (SPF) by means of a flowing liquid interface. The flowing liquid was ethanol which picks up the solute in the effluent of the gas chromatograph and this alcohol solution was monitored in a flow cell at the desired excitation and emission wavelengths. They have used this setup to measure pesticides, air pollutants, and methylenedioxyphenyl compounds. The detection limits (in nanograms) for various PCAHs were reported as fluorene (0.9), anthracene (10.0), p-terphenyl (0.4), chrysene (9.0), and BAP (0.7). However, the recoveries of substances by this method were not quantitative and the procedure was limited to fluorescing compounds only. Davis (98) reported the use of an EC detector with helium glow discharge as the electron source, which was capable of operation up to 400°C. Using this detector, Davis (98) claimed the detection of as little as one nanogram of BAP in cigarette smoke and showed that the analyses by GLC were in good agreement with those of a fluorometric method. Duncan (101) further illustrated the use of an EC detector in the determination of BAP emitted by industrial processes and

combustion effluents. He also reported the use of a mixture of sodium chloride with the liquid phase SE-30, on chromosorb G to separate BAP from BEP and benzo(k)fluoranthene, using an EC detector. The analysis time after extraction of the air sample was about 15 minutes. He further gave a list of 18 different columns, with different lengths and material, packed with five different liquid phases on a variety of solid supports (SE-30, SE-52, F.F.A.P, P.M.P.E, and Dow 200), that were tried for the separation of BAP from BEP.

A combination of pyrolysis and GLC to volatilize and separate for identification, the organic components of roadway dust was described by Thompson (52, 53). By this procedure he identified styrene from styrene-butadiene rubber in roadway dusts. Todd (66) has used this technique in the identification of PCAHs from "furnace thermal" carbon black. The pyrolysis GLC is a fingerprinting technique and has a great potential in the analysis of nonvolatile materials such as proteins, some drugs, bacteria, etc. Juvet (102), in a recent review, selected 742 references dealing with technique centered aspects of GLC during the years 1968 to 1969. According to Juvet, the American Chemical Society has reported from the 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."

Recently, Searl et al. (103) demonstrated a combined use of GLC and ultraviolet absorption spectrometry in the analysis of PCAHs from coke oven effluents. Using this technique they identified and quantitated F, pyrene, 1.2-benzanthracene, chrysene, BAP, and BEP in

coke oven effluents. They used a 10' x 0.125", stainless steel column packed with 2 per cent SE-30 on chromosorb G (80/100 mesh, acid washed and dimethyl silyl ether treated). In a very recent study, Burchfield et al. (104) investigated the development of a gas phase fluorescence detector for the analysis of PCAHs by GLC. They connected a Micro-Tek Model MT-160 gas chromatograph (Tracor, Inc., Austin, Texas) equipped with a 13.5 mCi ^{63}Ni electron capture detector in series with an Aminco-Bowman spectrophotofluorometer. PCAHs, after being separated on GLC column (6' x 0.25", glass-column packed with 10 per cent Dexil 300 and coated on 80/100 mesh chromosorb W) and measured by the EC detector, were then passed on through a heated transfer line into a microflow cell where the compounds were measured by fluorometry.

Identification and quantitation

After separation and detection, the individual PCAHs can be identified and quantitated by a number of methods available. The choice of a method depends on a number of factors such as: a) quantity of sample, b) type and kind of instrument available, c) selectivity (or specificity) of the instrument, d) sensitivity and accuracy of the method, e) reproducibility of the results, f) man-hours needed for the analysis, g) expertise of the analyst and h) cost of analysis. In the past, several procedures have been used for the identification of PCAHs. Some of these earlier methods of PCAHs analysis have been reviewed by Smith (54). The more recent and most widely used methods are:

a) Ultraviolet absorption spectrometry,

b) Luminescence analysis,

c) Gas liquid chromatography, and

d) Miscellaneous newer but less popular techniques.

Ultraviolet light is absorbed by all polycyclic aromatic compounds. The degree of absorption of light energy is a characteristic of a compound that may be used for the identification and determination of its concentration in a medium. The measurement of such absorption has been widely used in PCAHs analysis from air pollution (26, 50, 73, 83, 91), carbon black (69, 72, 95, 99, 100), coke oven effluents (102), processed rubber (51, 54), smoked foods (76, 77, 78, 84), and others (75, 86, 92). The advantages of this technique include the commercial availability of high quality UV spectrophotometers, the relative insensitivity to absorption by many trace impurities, and the high degree of sensitivity for the PCAHs. It is possible to determine and quantitate microgram amounts of PCAHs by UV absorption spectrophotometry. The disadvantages include the requirement of at least two or more separatory steps for reliable identification and quantitation, and relatively lower detection sensitivity as compared to fluorometric analysis.

Luminescence analysis is a most powerful, most sensitive, and most widely used analytic technique today. Almost all PCAHs are capable of absorbing light energy (ultraviolet and visible) and then releasing the excess energy by way of fluorescence and phosphorescence. (See Appendix B for definitions.) Fluorescence or phosphorescence emission occurs at a longer wavelength than the absorbed energy. Both wavelengths are specific for a compound and can be used for qualitative analysis. The relative intensity measurement is used for quantitative analysis, since the fluorescence intensity is directly proportional to

the concentration of the fluorescing compound while other factors are held constant. The high specificity of SPF helps to simplify and increase reliability of the analytical technique because a compound often may be measured directly without any prior separation (105) The most useful instrument for luminescence analysis is the spectrophotofluorometer (SPF).

Sawicki et al. (106) identified BAP unequivocally in a mixture of 50 PCAHs by fluorometric analysis. According to them the minimum amount of BAP detectable using fluorescence is a part per billion. Similarly, they were also able to detect BEP, perylene, and anthanthrene in the presence of other PCAHs. Fluorometric analysis has been extensively used in PCAHs identification and quantitation in air pollution (26, 37, 46, 80, 81, 83, 85, 86, 87, 91, 101), high boiling petroleum distillates (89), smoked foods (76, 77, 78), rubber tire dust, and carbon black (64, 66). More recently, the high specificity and sensitivity of SPF is being employed as a detector for sensing and identifying PCAHs in gas chromatography (97, 104). The disadvantages of luminescence analysis are that an analyst is required to control directly many instrumental variables and no standard method of obtaining and reporting luminescence spectra is available.

The gas-liquid chromatography is the most sophisticated technique which combines the principles of separation, detection, and identification. Despite earlier limitations, it shows increasing promise in qualitative and quantitative analysis of PCAHs. Mixtures of PCAHs may be separated and detected at nanogram levels if proper attention is given to the selection of columns (liquid phases), detectors, and opera-

ting conditions. Gas chromatographic analysis has been used in identification and quantitation of PCAHs from soot (95, 99, 100, 107), cigarette smoke (94, 98), rubber tire dust (52, 53), carbon black (66), coke oven effluents (103), and air pollution (93, 96, 101). Very recently, Bowman and Beroza (97) and Burchfield et al. (104) introduced fluorescence detectors in gas chromatography. This new development has not only enhanced the detecting powers of GLC but has also increased its sensitivity and selectivity to a great extent. Its advantages are quite obvious and include: a) fast speed of analysis, b) no prior separation steps for soot, cigarette smoke, air pollutants, carbon black, etc., c) separation, detection, identification, and quantitation are achieved at the same time, and d) it is available at a moderate cost. Proper selection of column, liquid phase, detector, and other operating conditions, however, takes considerable time. Further availability of authentic standard samples is limited. The best use of gas chromatography is in conjunction with other confirmatory spectrophotometric techniques.

Miscellaneous instrumental techniques include infrared and Raman spectroscopy, mass spectrometry, nuclear magnetic resonance, correlation spectroscopy, and electron spectroscopy. In 1968, Thomas et al. (65) used infrared analysis to describe the fate of BAP, perylene, and BEP in the atmosphere. The low sensitivity and nonspecific nature of infrared makes it unsuitable for trace analysis of PCAHs such as those found in urban air. No special techniques have been developed for infrared analysis of polycyclic compounds.

Keefer et al. (108) reported the use of nuclear magnetic

resonance spectrometry (NMR) for the analysis of mixtures of isomeric polynuclear hydrocarbons. NMR analysis is particularly very useful for PCAHs derivatives such as 1-methyl-, 2-methyl-, or 4-methylpyrenes; methyl anthracenes, phenanthrenes, and other methyl derivatives which are very hard to separate and quantitate by other techniques. According to these authors, the lower limit for NMR analysis is 30 to 40 micrograms of each component in the mixture. Therefore, it is also relatively insensitive and unsuitable for trace analysis.

Mass spectrometry is practical over a range of sensitivities somewhat greater than those available with the more convenient and less expensive ultraviolet spectrophotometers and spectrofluorometers. Mass spectrometry is based on high-vacuum ionization of organic molecules by bombardment with 70 electron-volt electrons. The ions thus generated under vacuum may be deflected and focused variously by electric or magnetic fields such that mass (inertial) differences allow the ions to be dispersed and displayed according to their own masses (38). Mass spectrometry offers a sensitive means of determining the probable identity and relative purity of PCAHs chromatographic fractions. The disadvantages include high costs, the requirement of some prior separation technique, and requirement of some skills for operating and interpreting the data. It is not suitable for routine trace analysis of PCAHs. Recent coupling of GLC with mass spectrometry suggests benefits in applying this sensitive and selective technique to the analysis of PCAHs.

Other techniques such as correlation spectroscopy and electron spectroscopy have not been fully explored as yet for the analysis of polycyclic aromatic hydrocarbons.

CHAPTER II

PURPOSE AND SCOPE

The identification and quantitative determination of carcinogenic substances plays an important role both in the study of cancer pathogenesis and in cancer prevention. Polycyclic aromatic hydrocarbons and especially benzo(a)pyrene occupy a significant place among carcinogenic substances because they were the first carcinogens to be discovered and are perhaps the most investigated. Above all, they do not represent naturally occurring substances and are the products of human activity at large. Moreover, their presence in the human environment has been established by their direct detection in the urban environment. Long and repeated exposure to carcinogenic PCAHs undoubtedly can induce cancer not only in the experimental animals but also in human beings. They constitute active agents of various tars, carbon black, rubber tire dusts, asphalt, pitches, mineral oils, shale oils, smoked foods, soot, waxes, automotive exhausts, paints, inks, etc., and have caused such well known occupational cancers as the classic chimney sweeps cancer, cancer of the scrotum in wax pressmen, mule spinner's cancer, bladder cancer in workers with dyestuffs, rubber, leather and leather products, and paint; and lung cancer from tar fumes. Therefore, the presence of PCAHs in the environment constitutes a public hazard.

The Department of Environmental Health at the University of Oklahoma Health Sciences Center has been concerned with the possibility of an occupational health hazard due to long exposure to carbon black and rubber dust. The department has also exerted great efforts in developing methods and techniques by which carcinogens can readily be identified and quantitated and their sources of production, their channels of dissemination, and their routes of exposure can adequately be ascertained. It has been found that carbon black and rubber tire dust produce no harmful effects in animals by ingestion, skin contact, or inhalation over the period studied (62, 63, 71). However, carbon black and rubber dust particles have adsorbed at least 3 to 4 PCAHs that are carcinogenic when eluted and administered to the laboratory animals. These findings, plus the earlier findings that there are quite a few PCAH carcinogens in urban environments and the fact that there is an increase in the incidence of lung cancer (and non-neoplastic lung diseases such as bronchitis and emphysema), necessitate the development of a more direct and rapid method of detection, identification, and quantitation of PCAHs.

A review of the literature has indicated that no individual carcinogenic PCAHs (such as BAP) can account for the causation of lung cancer and that noncarcinogenic hydrocarbons may act as co-carcinogen, promoting agents, or even as an inhibitor (9, 22, 24, 25, 37). This means that all the PCAHs, carcinogenic as well as non-carcinogenic, should be identified and quantitated in the air, carbon black, rubber dust, and other sources. In the past, the identification of PCAHs from

these sources has involved an extraction procedure followed by two or more procedures of chromatographic separation. The separations were undoubtedly long and tedious and very often yielded only a very small quantity for spectral analysis. Also most of these methods were developed for the determination of BAP only.

The more recent development in GLC, especially in liquid partitioning phases and detection systems, suggested a further exploration of this technique for the analysis of PCAHs (37, 100, 101, 103). Therefore, the major objectives of this particular investigation were:

a) to develop a rapid and more direct method for separation and detection of all PCAHs in a semi-reinforcing furnace (SRF) carbon black;

b) to identify all the PCAHs so found; and

c) to quantitate all of the identified PCAHs adsorbed on the SRF carbon black.

CHAPTER III

MATERIALS, INSTRUMENTS, AND PROCEDURES

Reagents

Benzene (Bz) and carbon disulfide (CS_2) were "Baker Analyzed" reagents obtained from J. T. Baker Chemical Company. Benzene was further purified by triple distillation, discarding the first and the last 10 per cent at each step. Carbon disulfide was distilled only once. Iso-octane (i-C_8) was 99 mole per cent from Phillips Petroleum Company. It was distilled once, discarding the first and last 10 per cent. Further purification of i-C_8 was achieved by passing through a column packed with activated silica gel obtained from Grace Davison Chemical, Baltimore, Maryland.

The carbon black used in this study was a Semi-reinforcing furnace (SRF) black. It was obtained from J. M. Hueber Corporation, Hueber Sample Number 0032, dated September 10, 1964. The particle size of this black ranged from 600 to 1,000 Å in average diameter and the surface area varies from 25 to 35 square meters per gram.

A weighed sample of this carbon black was extracted with purified Bz in a Soxhlet extractor for 24 hours. The extract was transferred into a volumetric flask and made up to the mark with Bz. A 50 ml sample of this extract was then evaporated to dryness on a Buchi

Rotavapor under vacuum and over a warm water bath. The residue was dissolved in about 5 ml of i-C_8 and used for injection into a GC for initial development of a gas chromatogram. Another sample of the Bz extract was similarly evaporated to dryness and the residue was dissolved in 2 ml of CS_2 and saved for later development of gas chromograms and for obtaining retention data.

The PCAHs used as standards in this study are given in Table 5 along with their sources. Unless otherwise stated, all ground glass stoppered with ground glass stoppers and ground glass connections were used for distillations, extractions, and for preparation of std solutions.

Standard solutions of the PCAHs for GC and SPF analysis were prepared in i-C_8. A known amount of PCAH was weighed and dissolved in a small volume of i-C_8. It was transferred quantitatively into a volumetric flask by rinsing the container at least four to six times with i-C_8 and finally diluting it to the mark with the solvent. For SPF and UV absorption analysis these PCAH standards (stds) were diluted further with i-C_8. The concentrations of SPF stds ranged from 0.1 to 2.5 ug/ml. The excitation (Ex) and fluorescence (Fl) spectra of each PCAH were recorded from 200 to 800 namometers (nm) on the spectrophotofluorometer.

The naphthalene, anthracene, and phenanthrene stds for GC analysis were also prepared in CS_2. The CS_2 standards were prepared by transferring a desired volume of previously prepared standard solutions (in i-C_8) into 50 ml Erlenmeyer flasks. The i-C_8 solution was evaporated to dryness on Buchi Rotavapor under vacuum and on a warm water bath. The flask containing the residual PCAH was rinsed four to five times with

TABLE 5

POLYCYCLIC AROMATIC HYDROCARBON
STANDARDS AND THEIR SOURCES

Hydrocarbon	Sources
Pyrene	Eastman Organic Chemicals
Chrysene	" " "
Fluoranthene	" " "
3-Methyl cholanthrene	" " "
1.2-Benzanthracene	" " "
Anthanthrene	K & K Laboratories, Inc.
1-Methyl pyrene	" " "
9.10-Benzophenanthrene	" " "
1.2,3.4-Dibenzpyrene	" " "
3.4,8.9-Dibenzpyrene	" " "
3.4,9.10-Dibenzpyrene	" " "
1.2,3.4-Dibenzanthracene	" " "
3.4,5.6-Dibenzophenanthrene	" " "
1.2-Benzopyrene	Aldrich Chemical Co., Inc.
Perylene	" " "
Naphthalene	" " "
Phenanthrene	" " "
o-Phenylene pyrene	" " "
1.12-Benzoperylene	" " "
9,10-Dimethyl anthracene	" " "
1.2,5.6-Dibenzanthracene	California Corporation for Biochemical Research
9,10-Dimethyl-1.2-benzanthracene	" " "
5,10-Dimethyl-3.4,8.9-dibenzpyrene	" " "
Coronene	Fluka AG, Buchs. SG, Switzerland
3.4-Benzopyrene	J. T. Baker Chemical Co.
Anthracene	Chemical Service Media
Naphthacene	City Chemical Corporation
1.2-Benzanthracene (Tetraphene)	Nutritional Biochemicals Corporation

CS_2, transferring each rinse into a volumetric flask. The final volume was made up with CS_2. The concentration of PCAHs in the std solutions ranged from 9 to 64.5 ug/ml. These stds were used to inject into gas chromatograph for retention time (t_R) measurement.

Instruments

Rotavapor R (Rotary Vacuum Evaporator)

An all glass evaporator, Model VE-50 Rotavapor R (Buchi) from Rinco Instrument Company, Inc., was used for evaporation dryness, and concentration of solutions of PCAHs. It consists of a rotating flask, receiving flask, condensing unit, motor and gear unit, long vapor duct with 24/40 joint, ball joint clamp, base, and stainless steel support rod.

Gas chromatograph

All gas chromatographic analyses were performed with the Hewlett Packard (F & M), Model 5750, Research Gas Chromatograph. It is a very versatile and sensitive instrument equipped with dual columns, dual thermal conductivity (TC), and dual flame ionization (FI) detectors. Because of extremely low sensitivity of TC detectors, they were not used for detection of PCAHs in this investigation. However, the exit ports of the TC detectors were used for collection of all the eluted peaks. The TC detectors received the column flow from a 50 to 50 effluent splitter housed in the oven (109). The FI detector has high sensitivity and permits the use of almost any carrier gas with the requirement that the effluent, or a fraction of it, carrying the separated PCAH components from the column be burned at a capillary tip in a

hydrogen-air flame. Intermediate combustion states of the organic molecule are ionized and appear as an electrical current between two polarized electrodes. The current is then amplified into an electrical signal suitable for display on a strip-chart recorder. The advantage of the FI detector is its wide dynamic range of linear response. It has a very low internal volume and an extremely low response time. These characteristics coupled with its high sensitivity and small sample requirements permit the ultimate in resolution (109).

The research gas chromatograph consists of a number of functional systems that were described by Todd (66) as well as by the GC Instruction Manual (109). A Hewlitt-Packard (Mosley), Model 7128A, strip-chart recorder was used. In addition to other controls, the electrometer drawer module has a range and attenuation control which is used in conjunction with the recorder. One 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 the range "1". Most of this chromatographic work was done with a range setting of "10", which is one-tenth as sensitive as "1", so that the full scale reading on the recorder was 0.05 volt (from Ohm's Law E=IR, and resistance equals to 5×10^{9} ohms). The attenuation allows the operator to reduce the electrometer output voltage to the recorder and thus prevents peaks from going off scale. An attenuation scale of "2" is half as sensitive as "1". In this work attenuation settings of "2", "4", "8", "16", and "32" were used. The higher settings were used only during collection of peaks.

Gas flow systems. Commercial high purity helium was used as

hydrogen-air flame. Intermediate combustion states of the organic molecule are ionized and appear as an electrical current between two polarized electrodes. The current is then amplified into an electrical signal suitable for display on a strip-chart recorder. The advantage of the FI detector is its wide dynamic range of linear response. It has a very low internal volume and an extremely low response time. These characteristics coupled with its high sensitivity and small sample requirements permit the ultimate in resolution (109).

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Gas flow systems. Commercial high purity helium was used as

the carrier gas. High purity hydrogen and compressed air were used for the flame ionization detectors. Helium and compressed air were purified by passing them through a molecular sieve, Type 5A, and a desiccant, packed in a 12 inch transparent acrylic tube which was obtained from Analab, Inc.

Chromatographic columns. The chromatographic column is the very heart of the instrument. The length and diameter of the columns chosen will be a function of the degree of resolution of separation needed, the analysis time, and the size of the sample which is to be injected. The two stainless steel columns (5' x 0.25" OD) chosen for this investigation were from Hewlett Packard. These columns were packed with 3 per cent silicone gum rubber OV-1 coated on 80/100 mesh Chromosorb W which was acid washed and treated with dimethyldichlorosilane (AW-DMCS). This treatment of the solid support greatly reduces its surface activity. The maximum recommended operating temperature for the column is 300°C. Both columns were fitted with splitter which divided the gas flow from the columns into two equivalent streams for simultaneous analysis by the two dual detector systems.

Gas liquid chromatography (GLC) is the most common form of gas chromatography used and is a process designed for the separation and analysis of gaseous and volatile materials. Like other forms of chromatography, gas liquid chromatography is basically a two-phase system (110). The stationary phase (liquid phase) is a stable, nonvolatile liquid at the column temperature, coated on a solid support. The solid support is a stable, inert material, such as diatomaceous earth, which provides a large surface area. The mobile phase is the carrier that may

be a liquid or a gas which pushes the sample through the column. In GLC the carrier is a gas, usually helium, nitrogen, or argon. Of these, helium is the most commonly used gas because of its small molecular size, high flow rate, high thermal conductivity, and inertness.

A sample is injected into the carrier gas, a plug of vapor is formed, and this is swept into the head of the chromatographic column. The components of the sample are immediately equiliberated between the liquid and mobile phases. They travel along the column according to their relative volatility and their physicochemical affinity for the liquid stationary phase at the temperature of operation. The concentration in each phase is governed by the partition coefficient (K)

$$K = C_l / C_m$$

where C_l and C_m are the concentrations of a component in the liquid phase and gas phase respectively. For example, when K = 1, the component will distribute itself evenly between the two phases and thus will spend half the time in the gas phase and half the time in the liquid phase, emerging at a retention time equal to twice the retention time of the air peak (111). Components will be separated only if they have different partition coefficients for a given gas-liquid system. If K for a component is small, the component has a larger amount dissolved in the mobile phase and will travel faster than a component with a larger K. Therefore, separation may be achieved and different components will be eluted and be detected at different time intervals after sample injection. The time intervals at which various components emerge are called retention times (t_R). The partition coefficient may be affected by a number of factors such as: a) vapor pressure of a component, b) solu-

bility of the component in the liquid phase, c) the size and symmetry of molecules of the component, and d) the polarity of the molecules.

Operating conditions of GLC. The chromatograms developed and retention times reported in this research were with the two 5' x 0.25", stainless steel, 3 per cent OV-1, coated on chromosorb W, AW-DMCS. The columns were attached at the "A" and "B" positions of a Hewlett Packard 5750 Research Gas Chromatograph. Helium was used as a carrier gas, and hydrogen and compressed air were used for the flame burner. The flow rate of the carrier gas was measured at the exit port of the TC detectors with a 10 ml bubble tube and a stop watch. As the splitter ratio was 50:50, the flow rate through the column was calculated by multiplying the observed flow rate by two. The hydrogen flow rate was determined by the difference between the helium plus the hydrogen flow rate at the tips of the burner (with the detector heater off), and the flow rate of helium alone measured at the tip of the burner. The details of pressure gauge settings and the flow rates are given in Table 6.

TABLE 6

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

Gases	Pressure Gauge Settings (lbs/in^2)	Flow Rate ml/min
Helium	42	120[a]
Hydrogen	16	48
Compressed Air	34	500[b]

[a]Average of 70 measured flows on 22 different days
[b]From Figure 4-1-3, page 4-1-5, Reference No. 109

Only flame ionization detectors were used for the development of chromatograms as they are 1,000 to 10,000 times more sensitive than good thermal conductivity detectors. The TC detectors were not used for detection during this work. However, the TC detectors and the TC oven were heated during the collection of peaks. The operating temperatures and various knob settings used for the development of chromatograms are listed in Table 7. The bridge current reading corresponding

TABLE 7

KNOB SETTINGS AND OPERATING TEMPERATURES (^{o}C)

	Knob Settings	Operating Temperatures (^{o}C) At Column Temperatures		
		200	225	250
Injection Port	6.5	335-340	360-365	370
FI Detector	8.5	340	348	350
Auxilliary	6.0	-	-	-
TC Detector	6.5	335-340	335-340	340
TC Oven	-	330	335	330
TC Auxilliary	5.0	325	325	325

to the temperature of the TC detector in Table 7 was 120 milliamperes. It was noted that with a change in column (oven) temperature, the temperatures of injection port and FI detector shifted upward without a change in their knob settings. The sensitivity of the FI detector may be increased further to a certain extent by higher gas flow rates. A higher hydrogen flow rate causes a rise in temperature and hence a greater degree of ionization.

Ten and fifty ul Hamilton syringes were used for all injections. The volumes of injected standard solutions and carbon black extracts in CS_2 ranged between 1 to 20 ul in size for normal development of chromatograms and for determining retention times of stds. Up to 50 ul of CS_2 solutions were injected during the collection of peaks.

Collection of peaks. Straight open-ended 17 cm x 0.4 cm, OD (with one end blown to 0.6 cm, OD) glass capillary tubes (Pyrex), containing a plug of silanized glass wool (previously washed with benzene and i-C_8), were used for collecting all eluted peaks. The narrow end of the capillary tube fits perfectly into the exit port of the TC detector when held straight as is shown in Figure 3. Most of the eluted PCAHs were coated on the inside of the capillary and approximately one centimeter from the exit port.

Spectrophotofluorometer

An Aminco Bowman Model 4-8203 spectrophotofluorometer (SPF) was used. It consists of a high intensity Xenon light source (Model No. A2-150N made by S. Antonino, Torino, Italy), a solid state blank subtract photomultiplier microphotometer (Model 10-280, from American Instrument Co., Inc.); a sample compartment; two monochromators; and a variable range X-Y recorder (Model 814A, made by Bolt, Beranck, and Newman, Inc.). The functioning of various parts of the instrument has been discussed by Snyder (64) and Todd (66) and also in the instrument bulletin (105).

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 adaptor. The

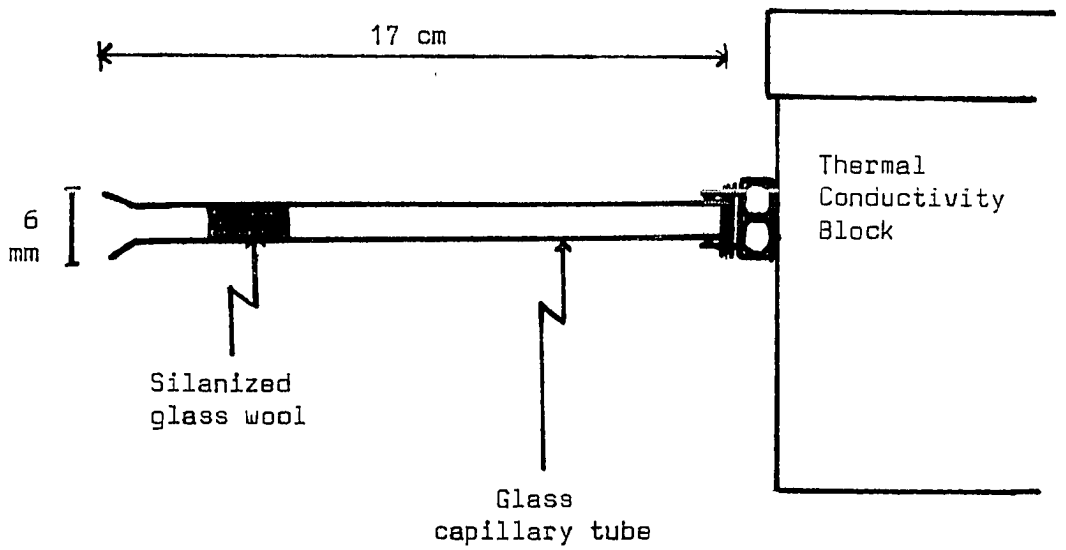

Figure 3.--Pyrex glass capillary tube for collection of chromatographic peaks.

excitation and fluorescence spectra recorded in this investigation were uncorrected with the following slit settings: A = 1, B = 0, C = 1, D = 1, E = 0, and F = 1 mm.

Ultraviolet absorption spectrophotometer

A Beckman, Ratio Recording Spectrophotometer, Model DK-2A, was used for the identification of some of the PCAHs. It has a single monochromator, double beam sample compartment, single source, and single detector system. It is equipped with a hydrogen lamp, hydrogen lamp power supply, tungsten lamp, tungsten lamp power supply, photomultiplier tube, detector, monochromator, and a lead sulfide cell detector. Details about the functions of various parts of this instrument are described by Willard et al. (111) and Trujillo (112).

Analytical Procedure

Identification and quantitation of PCAHs in a sample of carbon black extract involved the following four distinct steps:

a) Production of chromatograms

b) Identification of separated peaks

c) Collection of peaks and identification by SPF and UV absorption analysis

d) Quantitation of total PCAHs in the carbon black extract.

Production of chromatograms

In the early part of this investigation, several injections (1 ul to 20 ul) of the 28 PCAHs std solutions were made into the 5' x 0.25", 3 per cent OV-1 column at 200°C, 225°C, and 250°C column temperatures. The gas flow rates were held constant throughout this study

and were as shown in Table 6. The injection port and detector temperatures were held at temperatures given in Table 7. Recorder chart speed was set at 0.50 in/min throughout the study. Average retention times were determined for all PCAHs and their elution patterns from the column were also established. With respect to BAP, a sensitivity better than 5 ug/ml was achieved, which was reported by Sawicki et al. (91) for the flame ionization detector.

Next, several injections (5 ul to 10 ul) of carbon black extract in CS_2 were made into the column at the conditions specified above and the chromatograms were developed. Chromatographic separation was achieved under isothermal conditions at present temperatures (200°C, 225°C, and 250°C). It was observed that the lower temperature gives good separation of tri- and a few of the tetracyclic PCAHs.

Identification of separated peaks

The basis of qualitative analysis in GLC is a comparison of the retention indices (retention times, retention volumes, etc.) of a pure substance with those of the unknown under similar conditions. This type of information has been utilized in the prediction and partial identification of some PCAHs in soot samples, coke oven effluents, and cigarette smoke (92, 94, 95, 98, 99, 100, 103). Realizing the uncertainty in the separation of some PCAHs, some workers have collected the peaks and subsequently identified them by using ultraviolet absorption spectroscopy (92, 95, 99, 100, 103). It was reported that retention times alone are not sufficient for establishing the identity of all the unknown PCAHs, since two or more closely related PCAHs may have the same t_R. Also,

ultraviolet analysis requires good separation and relatively it is insensitive to very low concentration of other PCAHs as impurities.

In this study, the identification of separated peaks was not only made by comparison of retention times but also by enhancement of a peak by the addition of a small amount of the suspected compound. It was done by injecting a small amount of the suspected compound plus the carbon black extract in CS_2 into the column and then measuring the height or area of that peak. A true enhancement was indicative of the compound injected along with the extract. Thompson (52) employed this method in the identification of rubber in roadway dusts.

Collection of peaks and identification by SPF and UV absorption analysis

A known volume of carbon black extract in CS_2 (40 ul) was injected into the 5' x 0.25", stainless steel, OV-1 column at 200°C and all eluted peaks were collected separately in 17 cm x 0.4 cm, OD glass capillary tubes containing a small plug of silanized glass wool. In a similar fashion four more injections of carbon black extract in CS_2 (40 ul each) were injected and the peaks were collected in the same capillary tubes as before. Each injection took about two hours in eluting all the peaks from the column and in regenerating the column for a subsequent run. Thus, a total of 200 uls of carbon black extract in CS_2 were injected and separated by GLC. Each capillary tube containing PCAH was rinsed first with about 15 ml of CS_2 and then with 45 to 50 ml of i-C_8. The washings were collected in 125 ml conical flasks. The contents of each flask were evaporated to dryness on a Buchi Rotavapor on a warm bath and under vacuum. The residue was taken into a small volume

of i-C_8 and the contents of each were quantitatively transferred into a separate small volumetric flask. The volumes of each sample were made up to the mark with i-C_8, analyzed by the SPF and UV absorption spectroscopy. The UV absorption spectrum was obtained by scanning the above i-C_8 solution from 600 to 200 nanometers. The identity of each compound was established by comparison of its spectrum with that of known compounds.

The sensitivity control on the microphotometer was set at 100 per cent for all determinations. The slits were set at A = 1, B = 0, C = 1, D = 1, E = o, and F = 1. The BAP standard solution in i-C_8 was then scanned with the fluorescence monochromator manually at one or several excitation wavelength setting(s) to find the fluorescent bands. The wavelengths at which maximum fluorescence occurred were first determined, and then with this setting for the fluorescence monochromator, the excitation monochromator was scanned manually and the maximum excitation bands noted. The maximum excitation (Ex) and fluorescence (Fl) wavelength settings were found to be 386 and 402 nanometers (nm), respectively. The Ex and Fl spectra for BAP were then recorded at these predetermined wavelengths. Figure 4 shows the Ex and Fl spectra for BAP in i-C_8. Similarly, the maximum Ex and Fl wavelength settings for the remaining standard solutions were determined and their spectra were recorded. All 20 peaks obtained from the gas chromatographic separation of injected carbon black extract as collected above were examined with the SPF. It was found that some of the collected peaks, i.e., peak no. 4, 11, 13, 15, 17, 19, and 20, contained more than one compound. The identity of various peaks was established by comparison with the SPF

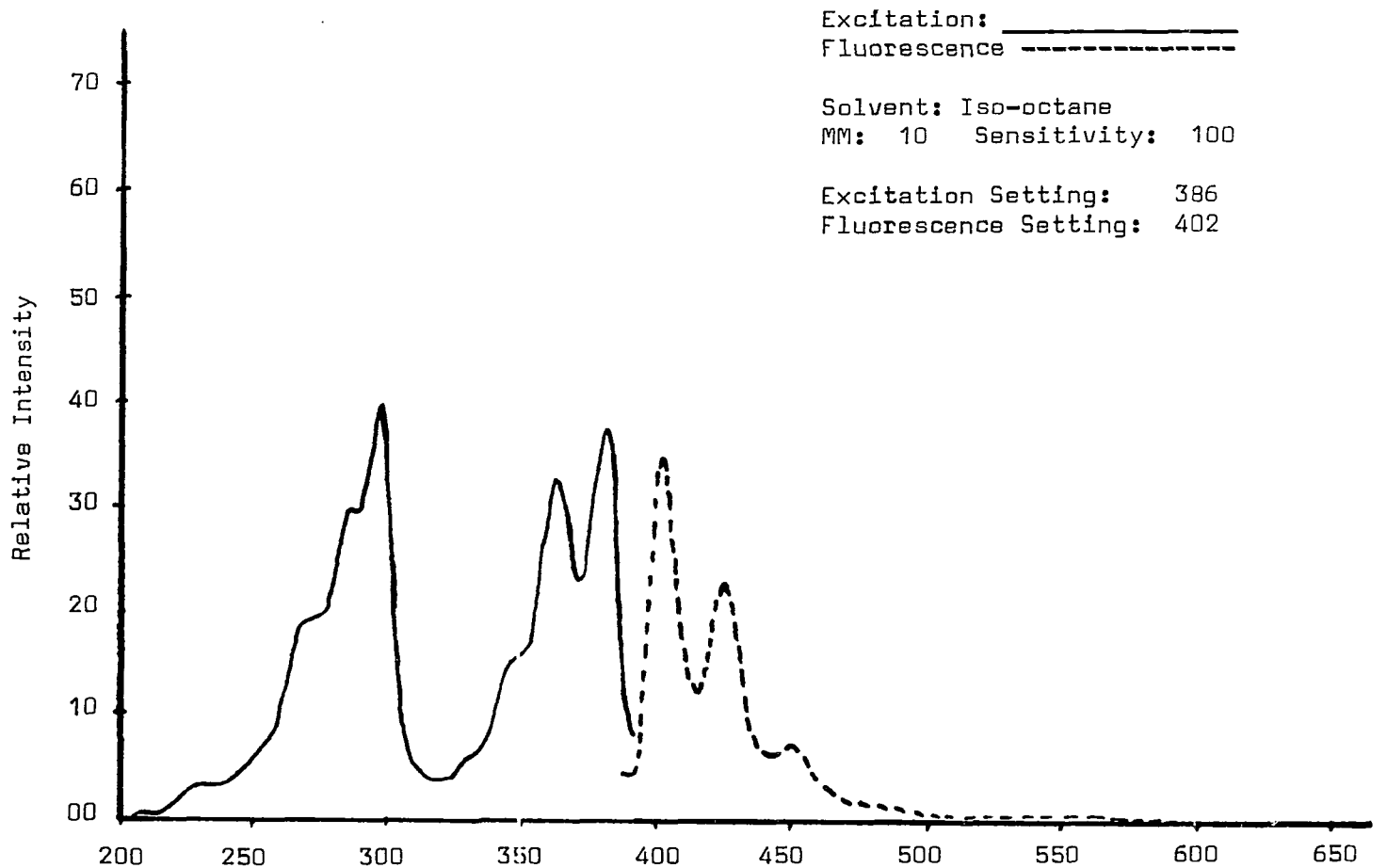

Figure 4.--Excitation and fluorescence spectra of BAP standard solution.

spectra.

Quantitation of PCAHs in carbon black extract

Quantitation of PCAHs in a carbon black extract was done by two methods since some chromatographic peaks were found to contain more than one component.

Standard plots of concentration (micrograms) against peak area (centimeter squares) were made for all the PCAHs separated. Peak area under those that had only one component was measured directly from the sample chromatogram. Knowing the volume of the sample injected, the quantity of each PCAH was then read directly from the corresponding plot.

The chromatographic peaks that have more than one component were collected as described before. The SPF spectra of chromatographic peaks 4, 11, 13, 15, and 17 were recorded at wavelength settings for two or more PCAHs. The SPF spectra of the suspected PCAH were recorded on the same graph at the same time. The PCAHs in these peaks were then quantitated from the peak height and the concentration of the corresponding PCAH.

CHAPTER IV

RESULTS AND DISCUSSION

Retention Times (t_R) of PCAHs and Gas Chromatograms of Carbon Black Extract

The average retention times in minutes of 26 PCAHs were determined on the 5' x 0.25", stainless steel, 3 per cent OV-1 column at three different temperatures and are given in Table 8. The t_R values for the PCAHs have been found to be reproducible on this chromatograph at any given temperature provided that all operating conditions are the same. Also the elution pattern or relative retention times of these PCAHs were the same at different temperatures. Therefore, the t_R values can be used for the identification of PCAHs in the carbon black extract.

Initially, the carbon black extract in CS_2 was injected into the gas chromatograph at the isothermal oven conditions of 200, 220, 225, and 250°C in order to determine the better temperature(s) for chromatogram development. It was found that the resolution (separation of peaks) was satisfactory at the lower temperature (200°C) while the response was poor for PCAHs with high t_Rs. There was also a great deal of tailing for these PCAHs at this temperature. At higher temperature(s), there was a good response and no tailing effect but the

TABLE 8

AVERAGE RETENTION TIMES OF POLYCYCLIC AROMATIC HYDROCARBONS

Hydrocarbon	Avg.* Retention Times (t_R) at Column Temp. 200°C (min)	225°C (min)	250°C (min)
Phenanthrene	1.35	1.11	-
Antrhacene	1.37	1.12	-
9- or 10-Methyl anthracene	2.29	2.00	-
Fluoranthene	3.02	-	-
Pyrene	3.26	2.09	-
9,10-Dimethyl anthracene	3.40	2.36	-
1-Methyl pyrene	5.20	3.04	-
9.10-Benzophenanthrene	9.00	4.46	2.18
Chrysene	9.14	4.56	2.30
1.2-Benzanthracene	9.16	4.58	2.30
2.3-Benzanthracene	10.40	5.30	2.39
9,10-Dimethyl-1.2-benzanthracene	22.14	11.12	4.24
1.2-Benzpyrene	26.04	12.16	4.30
3.4-Benzpyrene	26.03	12.24	4.40
3.4,5.6-Dibenzophenanthrene	26.18	12.36	4.42
Perylene	27.36	12.42	4.48
3-Methyl cholanthrene	-	16.10	6.03
1.2,3.4-Dibenzpyrene	-	23.18	7.46
1.2,3.4-Dibenzanthracene	-	24.03	8.40
o-Phenylene pyrene	57-59	24.52	8.50
1.2,5.6-Dibenzanthracene	-	25.24	8.58
1.12-Benzoperylene	60-62	27.12	9.58
Anthanthrene	63-66	29.06	11.02
Coronene	-	-	21-24
3.4,9.10-Dibenzpyrene	-	-	26-28
3.4,8.9-Dibenzpyrene	-	-	29-32

*Average of three injections of increasing concentration of each PCAH

resolution was very poor, especially for PCAHs with low t_R values. For better results, therefore, chromatograms were developed at isothermal oven conditions of 200 and 250°C. The chromatograms of carbon black so developed are shown in Figures 5 and 6, respectively. A 10 ul of CS_2 solution injected represents a weight of 1.64 mg of carbon black. The peaks are numbered from 1 to 20 and all were collected for further analysis by SPF and ultraviolet spectroscopy.

Identification and Confirmation of PCAHs in Chromatographic Peaks of Carbon Black Extract

The t_R values of PCAHs were used for the initial identification of compounds in the chromatographic peaks of carbon black extract. Thus, the compounds in the peak number (no) 4, 6, 7, 8, 10, 11, 14, 15, 16, 17, and 18 were tentatively identified by their t_Rs. Compounds in the peak no 1, 2, 3, 5, 9, 12, 13, and 20 were not identified simply because: a) the t_R values do not match with any of the available PCAH standards, b) they are present in trace amounts and would require a collection from a large number of sample injections, and c) corresponding PCAH standards were not available at that time. Peak number 19 had a retention time corresponding to 3.4,9.10-dibenzpyrene and was not clearly visible on some chromatograms.

Thompson (52, 53) confirmed the presence of styrene in rubber tire and roadway dusts by spiking of the sample with styrene or an enhancement of peak technique. This technique was used and the compounds in peak no 6, 7, 14, 16, and 18 were confirmed as fluoranthene, pyrene, 9,10-dimethyl-1.2-benzanthracene, o-phenylene pyrene, and coronene. The enhancement of peak 14 by 9,10-dimethyl-1.2-benzanthracene is shown in

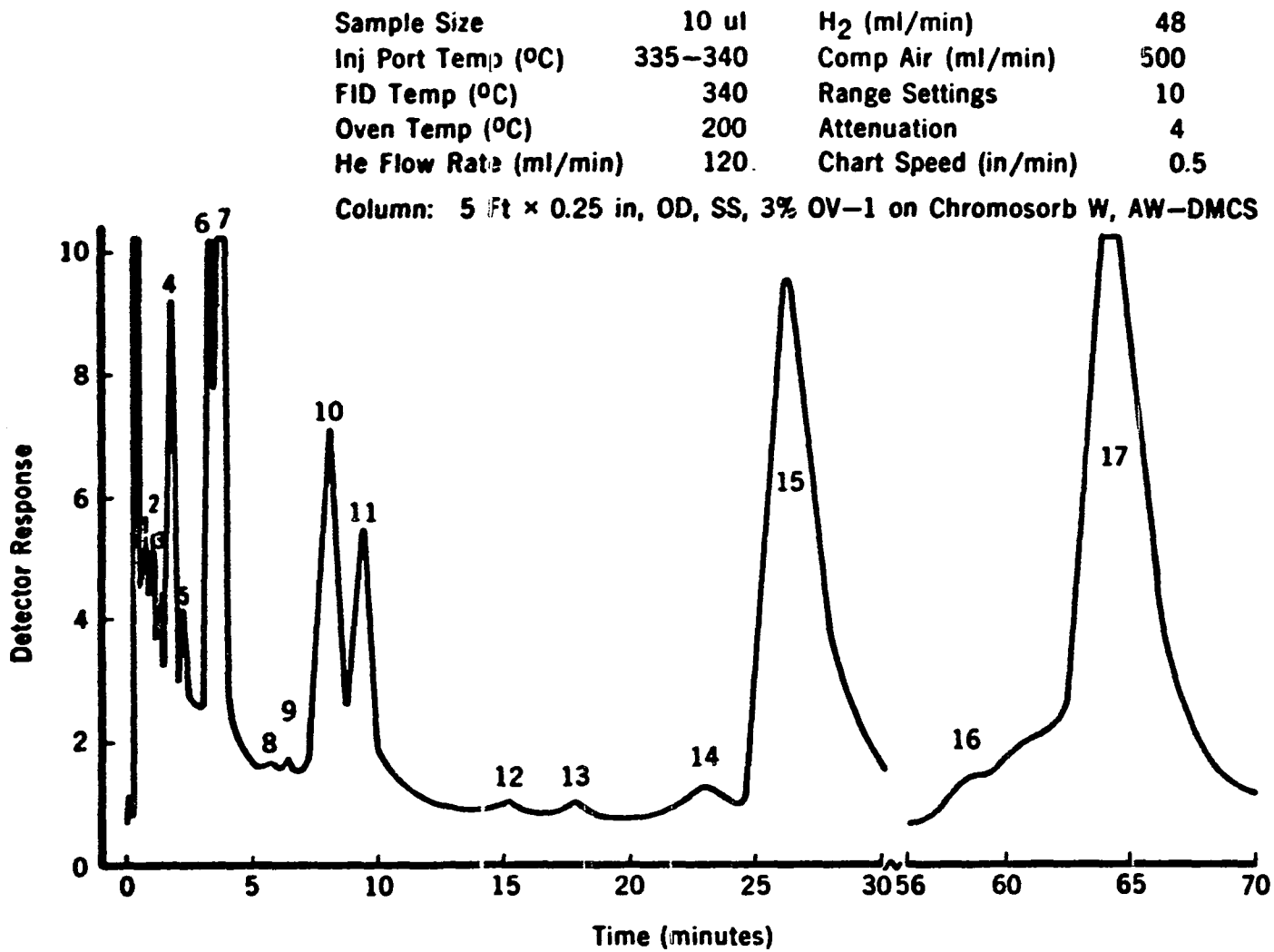

Figure 5.-- Gas Chromatogram of Polycyclic Aromatic Hydrocarbons from SRF Carbon Black with Flame Ionization Detector. Peak numbers corresponds to the compounds listed in table 9.

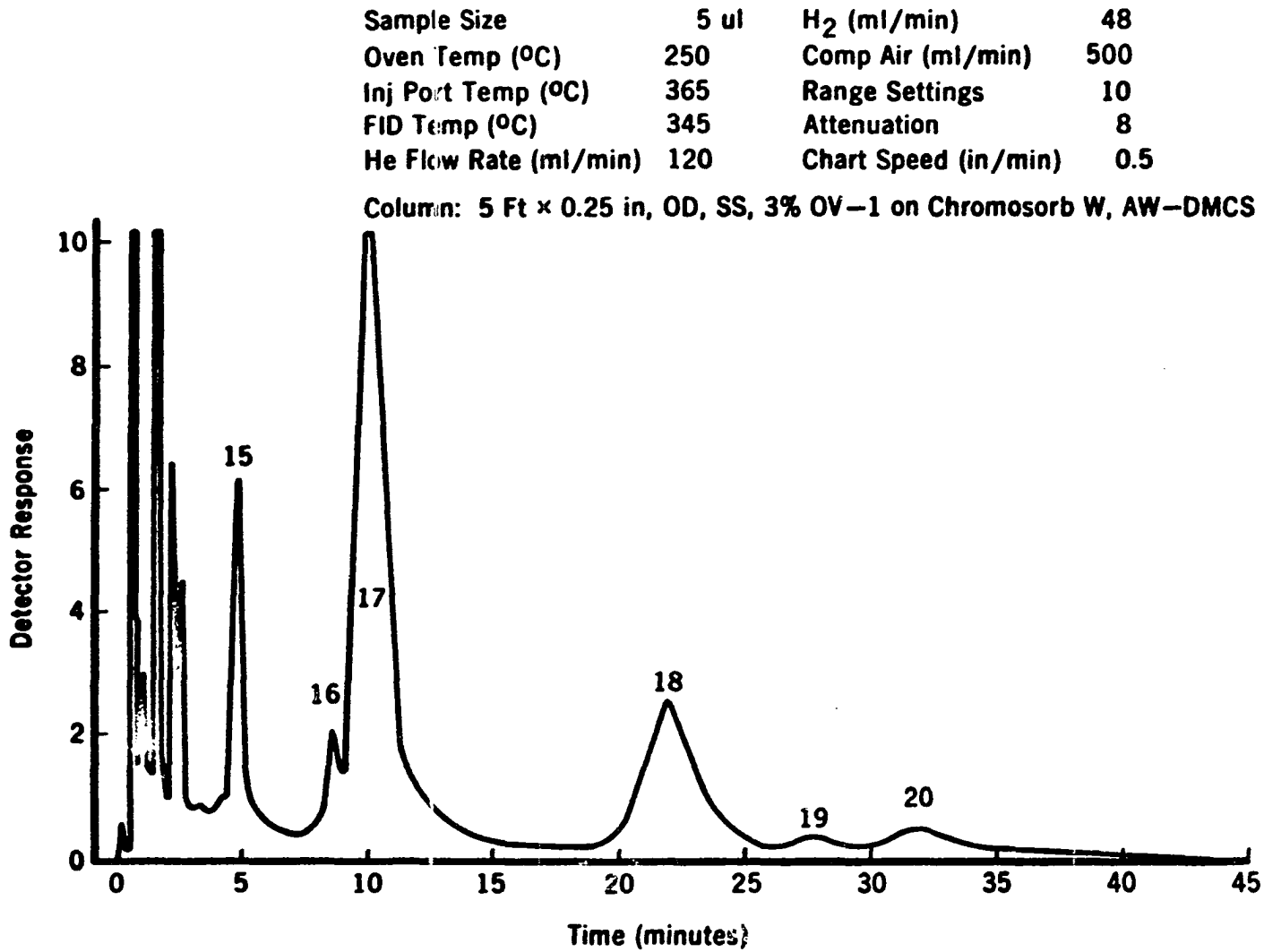

Figure 6.-- Gas Chromatogram of Polycyclic Aromatic Hydrocarbons from SRF Carbon Black.

Figure 7 by a broken line. It was also shown by SPF analysis that peak no 4, 11, 15, and 17 contained more than one compound. The compound in peak 10 was confirmed by a method similar to that used by Chakraborty and Long (95.) Peak no 10 was collected and its ultraviolet spectrum on comparison was found to be similar to that reported for benzo(mno)-fluoranthene by Clar (30).

Searl et al. (103) reported the incomplete separation of PCAH pairs 1.2-benzanthracene and chrysene, and BEP and BAP from coke-oven effluents on a 2 per cent SE-30 coated on Chromosorb G using a stainless steel column. Davis (98) reported some difficulty in complete separation of BEP, BAP, and perylene from cigarette smoke even with an electron capture detector and on a 3 per cent SE-30 column. Chakraborty and Long (95) had previously shown the incomplete separation of the following PCAHs from soot on a 10 per cent SE-52 column and temperature programming: anthracene and phenanthrene; 1.2-benzanthracene and chrysene; benzo(b)fluoranthene and benzo(k)fluoranthene; and BEP, BAP, and perylene. They collected the chromatographic peaks containing PCAHs and identified these by ultraviolet spectrophotometry. They have reported positive identification of at least 18 PCAHs. There was some question regarding the identity of peak no 7 (Chakraborty and Long's work) by Thomas and Monkman (107), which in a later work by Long and Chakraborty (99) was believed to be a mixture of 1- and 4-methylpyrene. Similarly, peak 9B (Chakraborty and Long's work) was regarded as a pyrene derivative in the original work, but Wallcave (100) has proved it to be cyclopenta(c,d)pyrene or acepyrene by mass spectroscopy.

Todd (66) reported the identification of 10 PCAHs from a fine

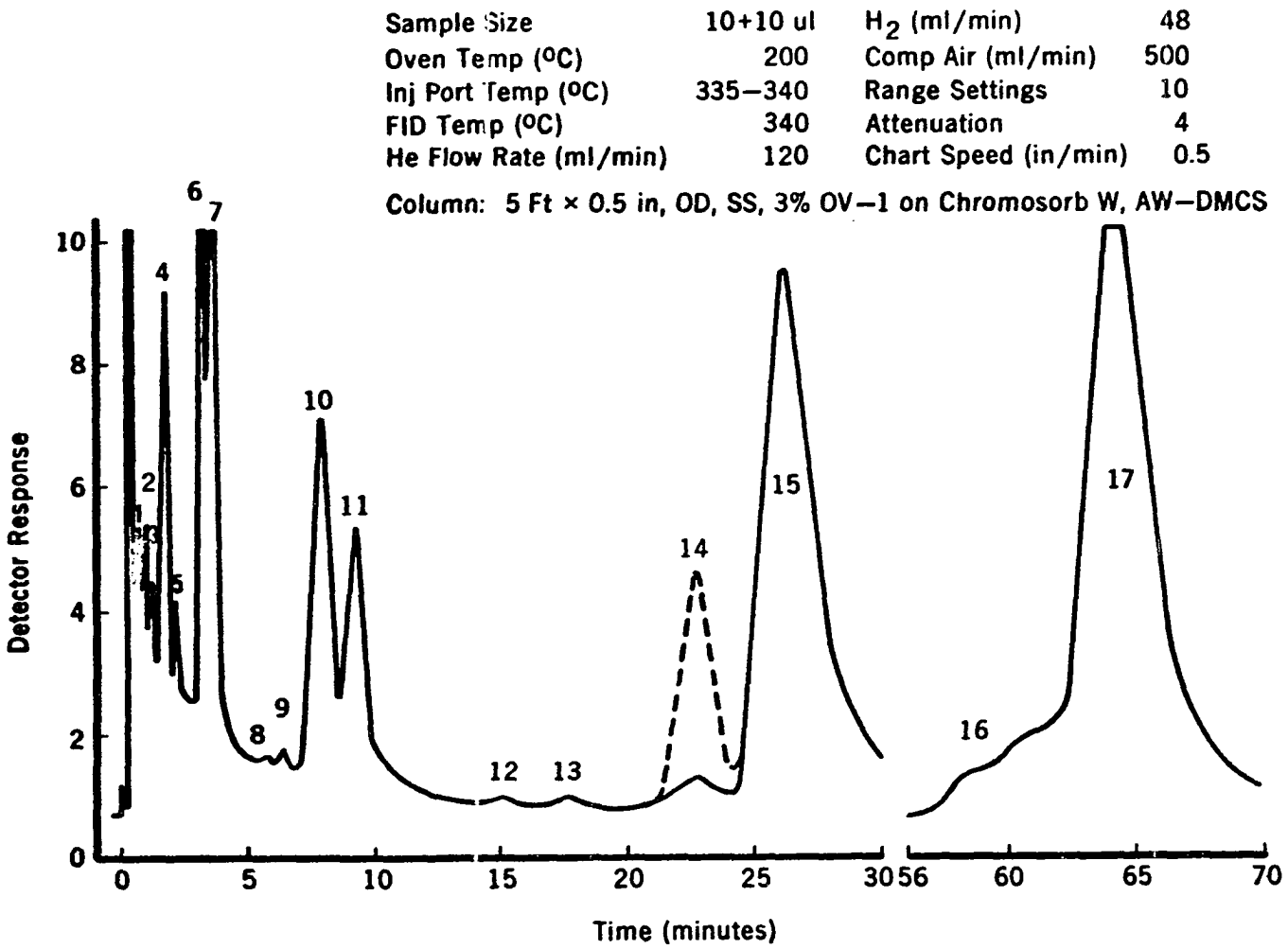

Figure 7.-- Identification of Chromatographic Peak 14 by Addition of a Suspected Compound (9, 10-dimethyl-1.2-benzanthracene).

"furnace thermal" black extract by SPF. He, too, had difficulty in separating 1.2-benzanthracene from chrysene, BEP from BAP, and 1.12-benzoperylene from anthanthrene on a 3.8 per cent SE-30 column. A comparison of the compounds identified in this investigation from SRF carbon black with those identified by Todd (66) and Chakraborty and Long (95) is made in Table 9.

The identification and confirmation of peak no 4, 11, 15, and 17 is similar to that reported by Todd (66). These peaks were collected separately and the presence in these peaks of anthracene and phenathrene; 1.2-benzanthracene and chrysene; BEP, BAP, and perylene; and 1.2-benzoperylene and anthanthrene, respectively, were confirmed by comparison of their Ex and Fl spectra with those of standard PCAHs. Further confirmation of compounds in peak no 6, 7, 14, 16, and 18 were also made by the comparison of their Ex and Fl spectra with those of known PCAHs.

Collection of Chromatographic Peaks and Identification of PCAHs in Carbon Black Extract

Although an attempt was made to collect all the peaks for complete identification and subsequent quantitation, some peaks such as 1, 2, 3, 5, 8, 9, 12, 13, and 20 either had so little amount of PCAH present or were so close to each other that not enough of each was collected to be able to make any positive identification of these peaks. Peak 4 was collected during a t_R of 1.24 to 2.0 minutes (min). Figures 8 and 9 show the Ex and Fl spectra of peak 4 when compared to those of anthracene and phenanthene standard solutions, respectively. It is quite clear that these two PCAHs are present in this peak. Todd (66) had reported it to be anthracene only in "furnace thermal" black. Peak no 6

TABLE 9

COMPARISON OF PCAHs COLLECTED FROM GAS CHROMATOGRAPHIC SEPARATION OF SRF CARBON BLACK EXTRACT TO IDENTIFIED PCAHs FROM CARBON BLACK AND SOOT SAMPLES

Peak No	PCAHs Detected	PCAHs Identified by Todd	PCAHs Identified by C & L[a]	C & L[a] Peak No
1	Unidentified	-	-	-
2	Unidentified	-	Acenaphthylene	1
3	Unidentified	-	Fluorene	2
4	Anthracene Phenanthrene	Anthracene	Anthracene Phenanthrene	3
5	Unidentified	Unidentified	Methyl Phenanthrene	4
6	Fluoranthene	Fluoranthene	Fluoranthene	5
7	Pyrene	Pyrene	Pyrene	6
8	Unidentified	Unidentified	1- & 4-Methyl-pyrene	7
9	Unidentified	-	-	-
10	Benzo(mno)-fluoranthene	Unidentified	Benzo(mno)-fluoranthene	8
11	1.2-Benzanthracene Chrysene	1.2-Benzanthracene Chrysene	1.2-Benzanthracene Chrysene	9A
12	Unidentified	Unidentified	Cyclopenta(c,d)-pyrene	9B

TABLE 9--Continued

Peak No	PCAHs Detected	PCAHs Identified by Todd	PCAHs Identified by C & L[a]	C & L[a] Peak No
13	Unidentified	Unidentified	Benzo(b)fluoranthene Benzo(k)fluoranthene	10
14	9,10-Dimethyl-1.2-benzanthracene	-	-	-
15	BEP BAP Perylene	BEP BAP -	BEP BAP Perylene	11
16	o-Phenylene pyrene	-	o-Phenylene pyrene	12
17	1.12-Benzo-perylene Anthanthrene	1.12-Benzo-perylene Anthanthrene	1.12-Benzo-perylene Anthanthrene	13 14
18	Coronene	Coronene	Coronene	15
19	Unidentified	-	-	-
20	Unidentified	-	-	-

[a]Chakraborty and Long

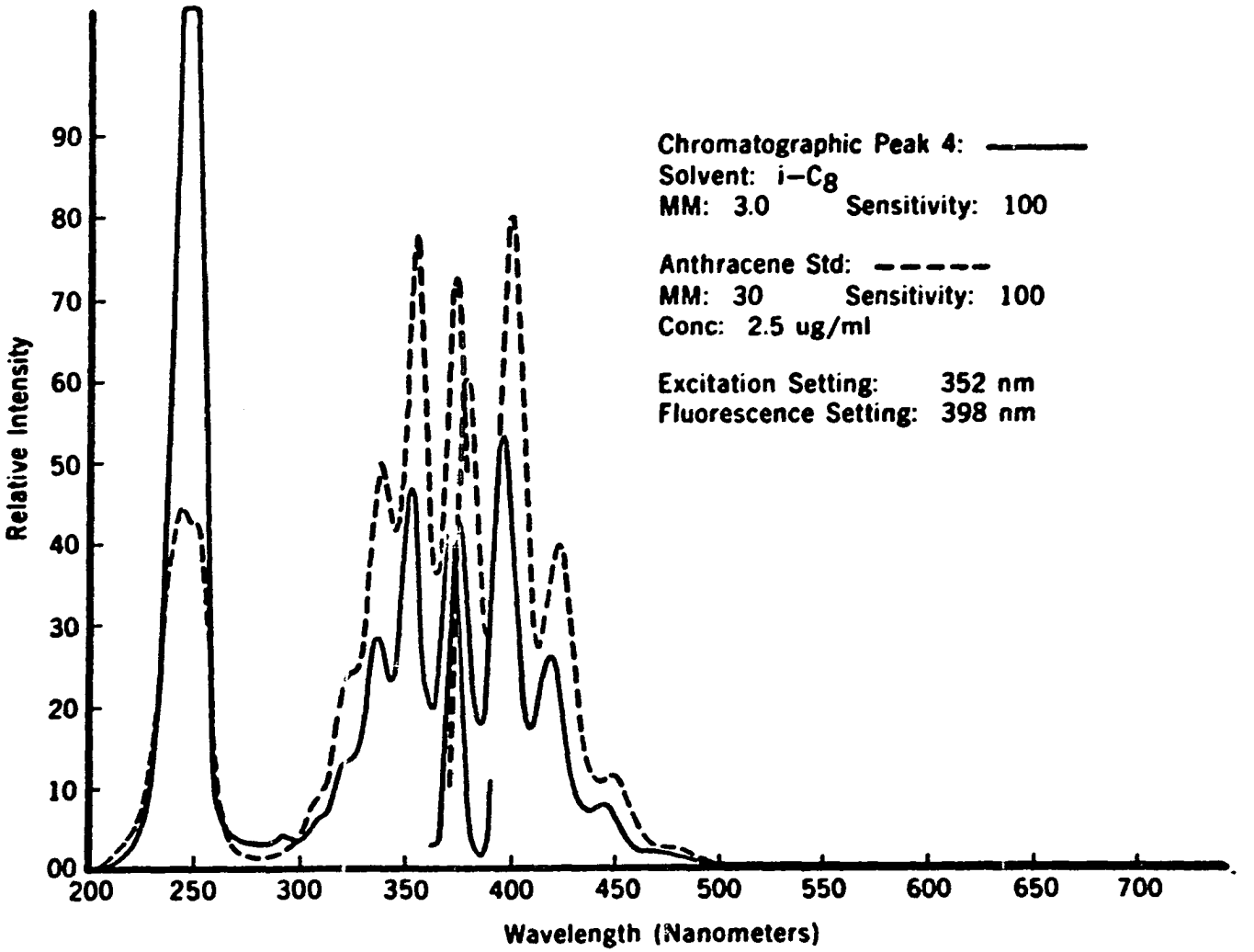

Figure 8.— Excitation & Fluorescence Spectra of Chromatographic Peak 4 from Carbon Black Extract and Anthracene in i-C_8.

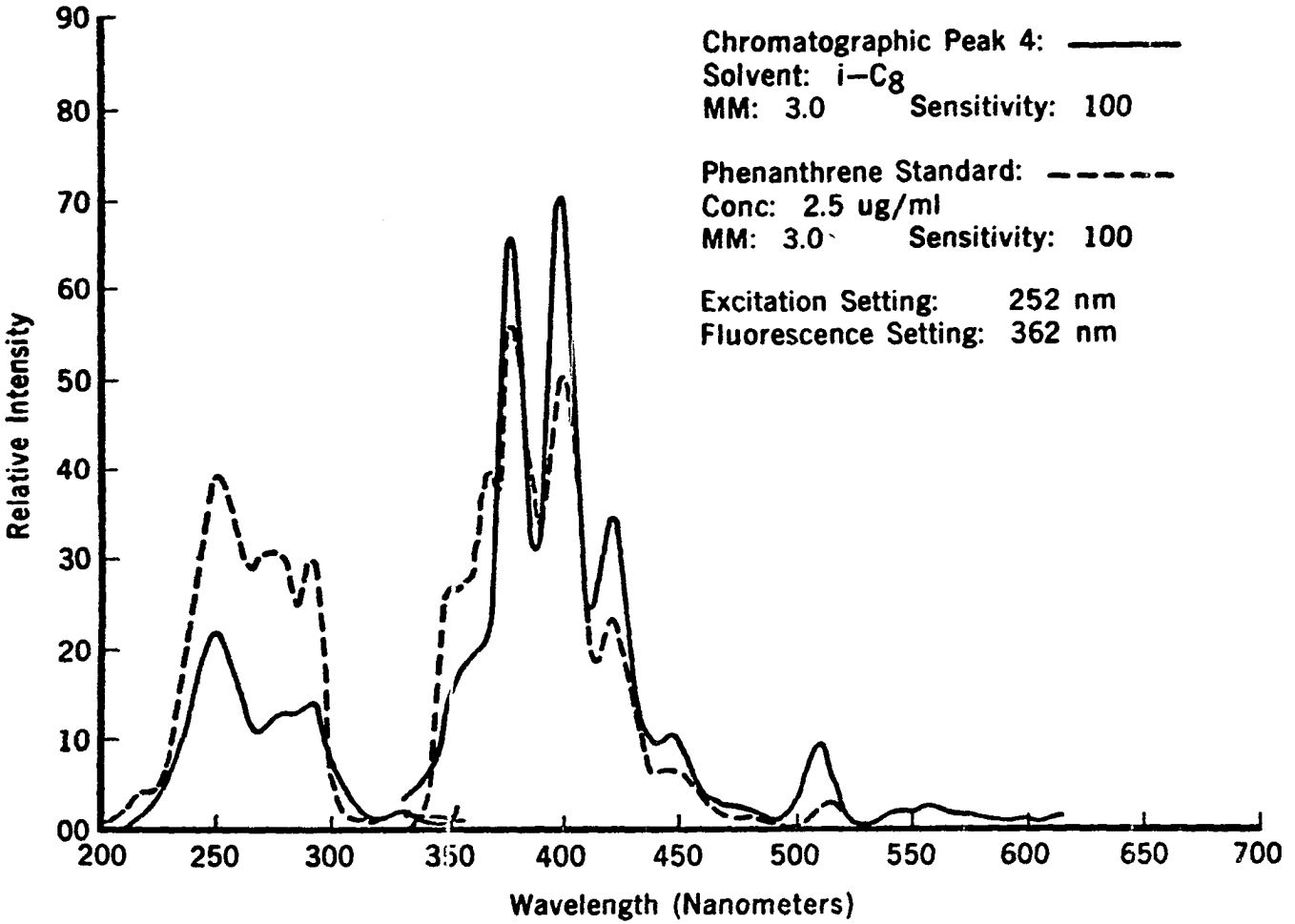

Figure 9.--Excitation & Fluorescence Spectra of Chromatographic Peak 4 and Phenanthrene in i-C_8.

and 7 were collected during a t_R of 3.0-3.30 and 3.36-4.18 min, respectively. Their SPF spectra in i-C_8 were recorded along with those of fluoranthene and pyrene stds, respectively. Chromatographic peak 10 was collected during a t_R of 7.24 to 8.36 min and its UV absorption spectrum was recorded. As mentioned before, it was identified by comparing its ultraviolet absorption spectra with that set of ultraviolet absorption spectra compiled by Clar (29, 30). It was found to be benzo(mno)fluoranthene. No benzo(mno)fluoranthene was available in the laboratory at that time. Figure 10 gives the ultraviolet absorption spectrum in i-C_8.

Chromatographic peak 11 was collected during a t_R of 8.36 to 10.0 min. Figures 11 and 12 compare the Ex and Fl spectra of peak 11 with those of chrysene and 1.2-benzanthracene std solutions, respectively. The SPF spectra in these figures are exactly similar to that reported for peak 7 by Todd (66). It is quite obvious then that both the chrysene and 1.2-benzanthracene are present in peak 11. Peak 14 was collected during a t_R of 21.0-23.36 min. Its identification was made by injecting a known amount of 9,10-dimethyl-1.2-benzanthracene along with the sample as is shown in Figure 7 by the broken line. Its presence was confirmed by comparison of its Ex and Fl spectra to that of a 9,10-dimethyl-1.2-benzanthracene std solution and is included in Appendix C.

Figures 13, 14, and 15 compare the Ex and Fl spectra of peak 15 with those of BEP, BAP, and perylene, respectively. It is clear from these figures that chrometographic peak 15 is composed of these three PCAHs. It was collected during a t_R of 24.0 to 30.0 min. The presence of BEP in this peak could be confirmed from its Ex spectrum. The Fl

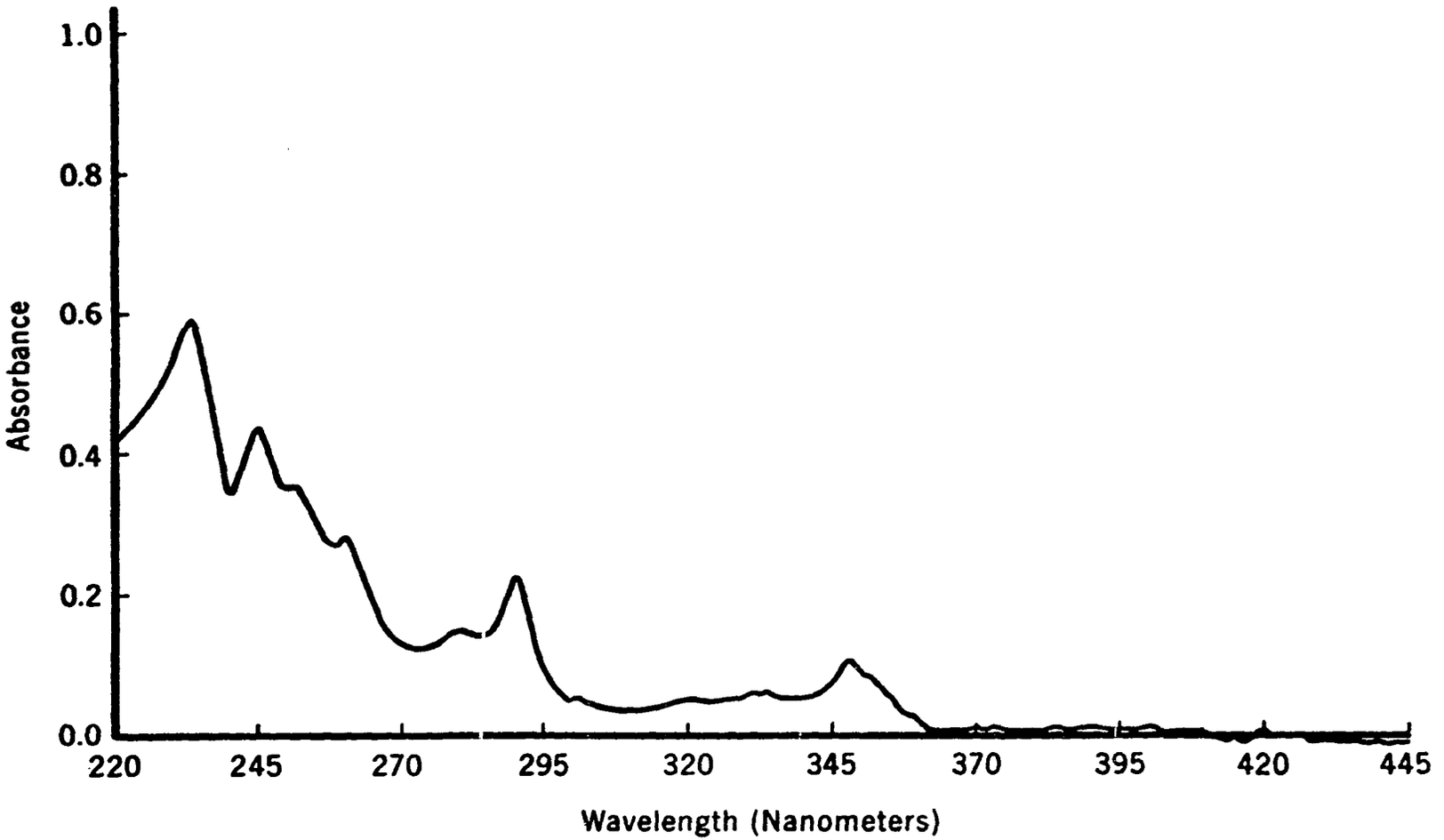

Figure 10.-- The Absorption Spectrum of Chromatographic Peak 10 in Iso-octane.

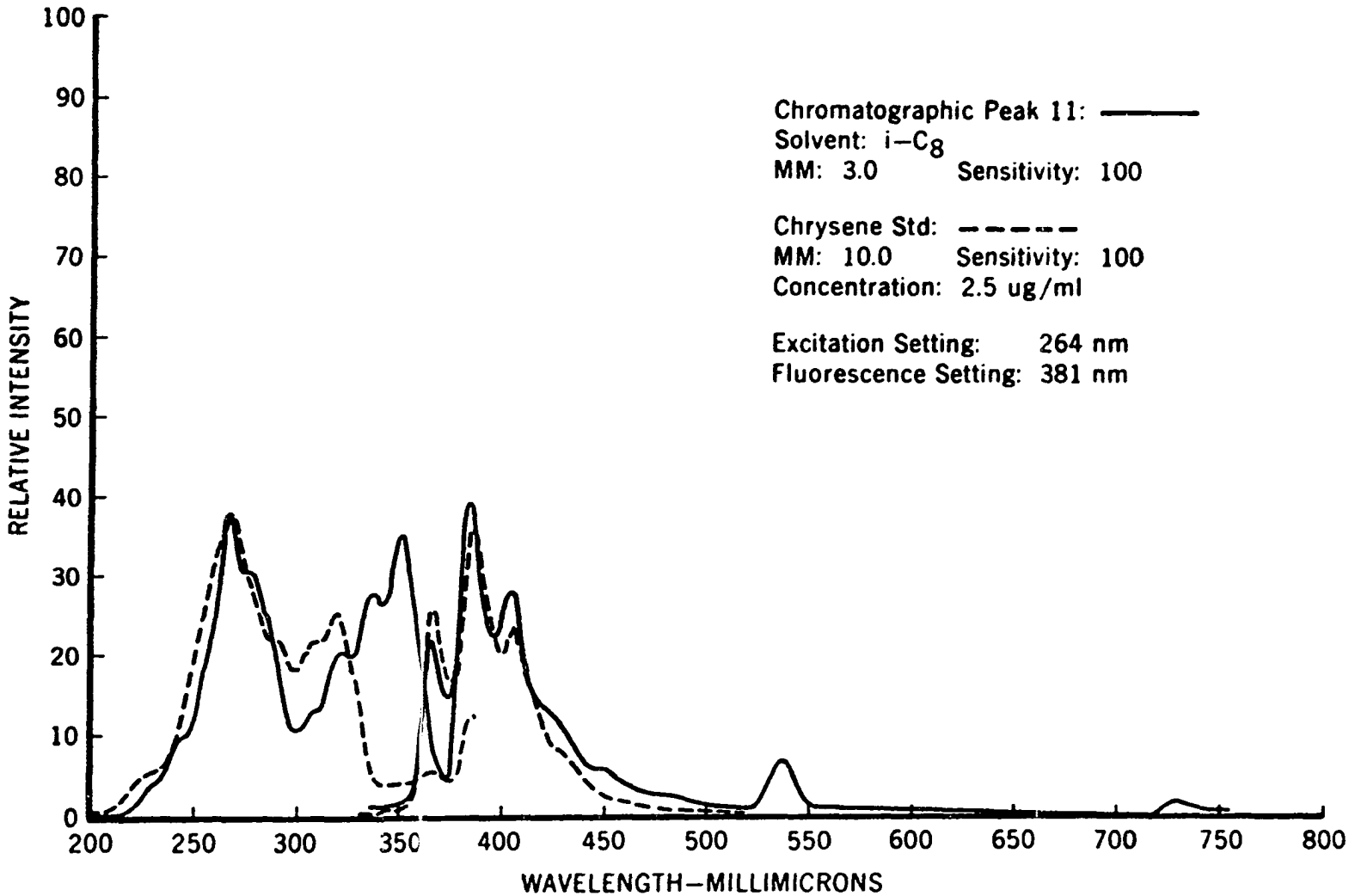

Figure 11.-- Excitation and Fluorescence Spectra of Chromatographic Peak 11 from SRF Carbon Black Extract and Chrysene.

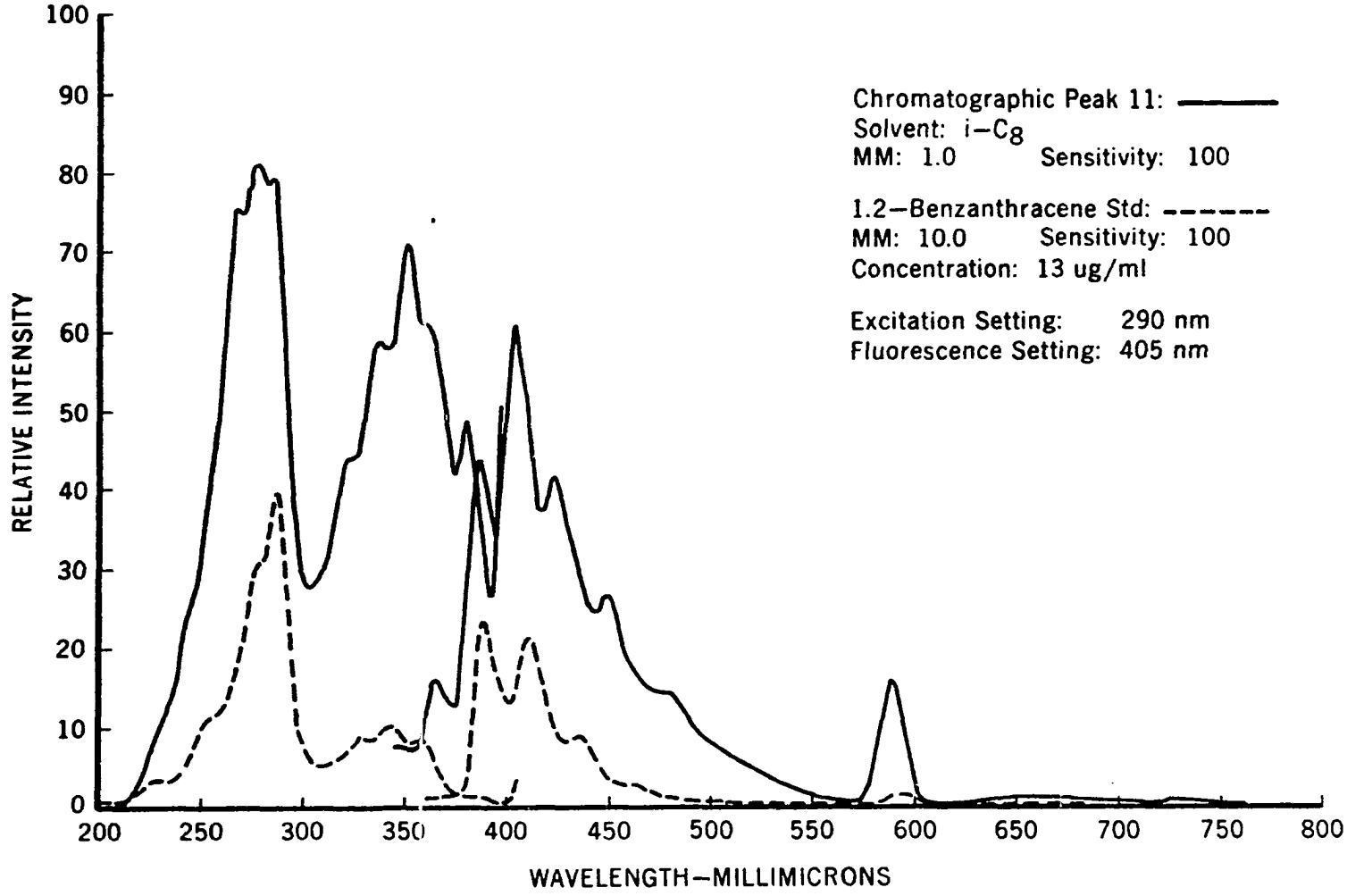

Figure 12.-- Excitation and Fluorescence Spectra of Chromatographic Peak 11 from SRF Carbon Black Extract and 1.2–benzanthracene.

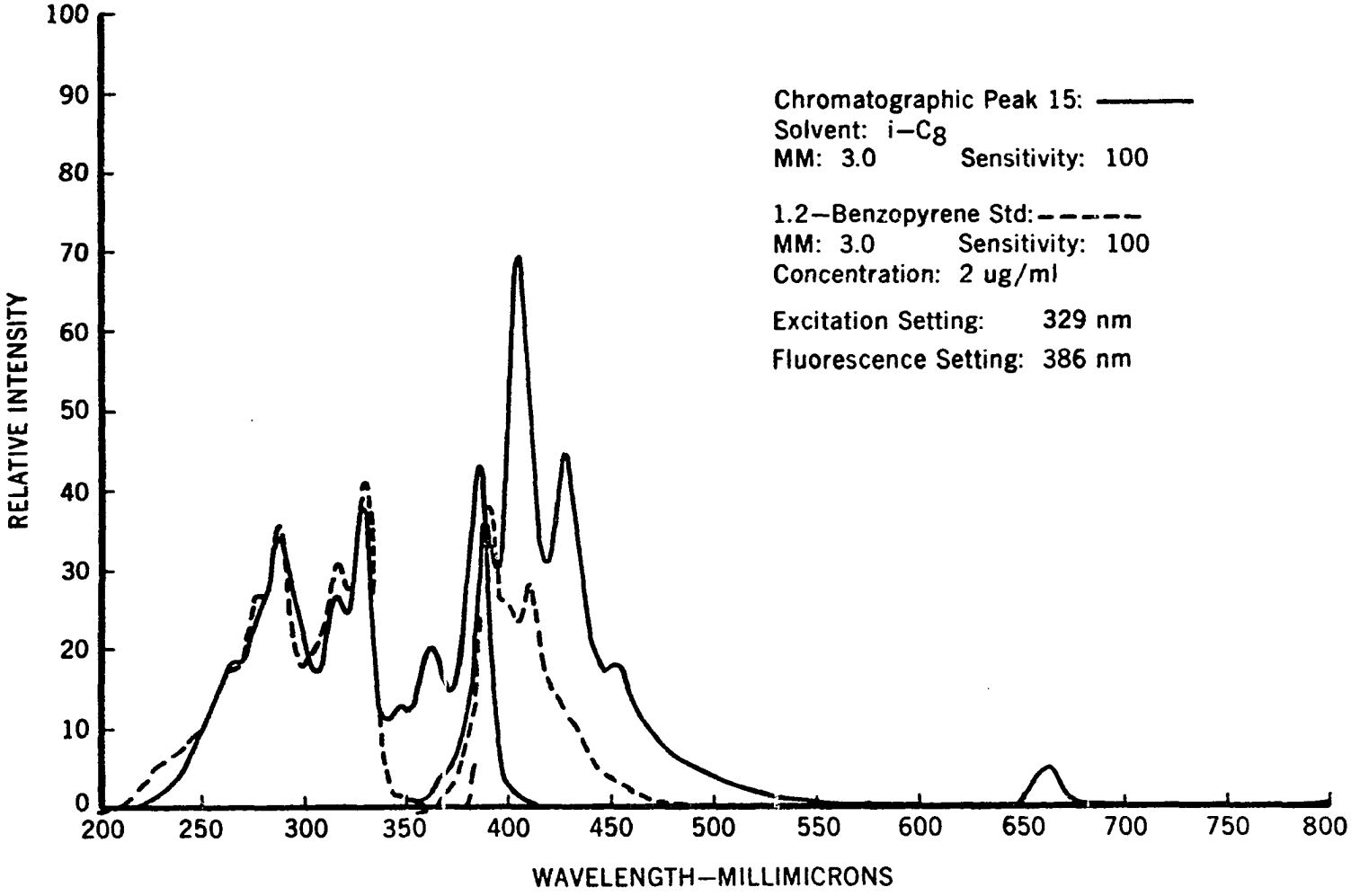

Figure 13.-- Excitation and Fluorescence Spectra of Chromatographic Peak 15 from SRF Carbon Black Extract and 1.2–benzopyrene.

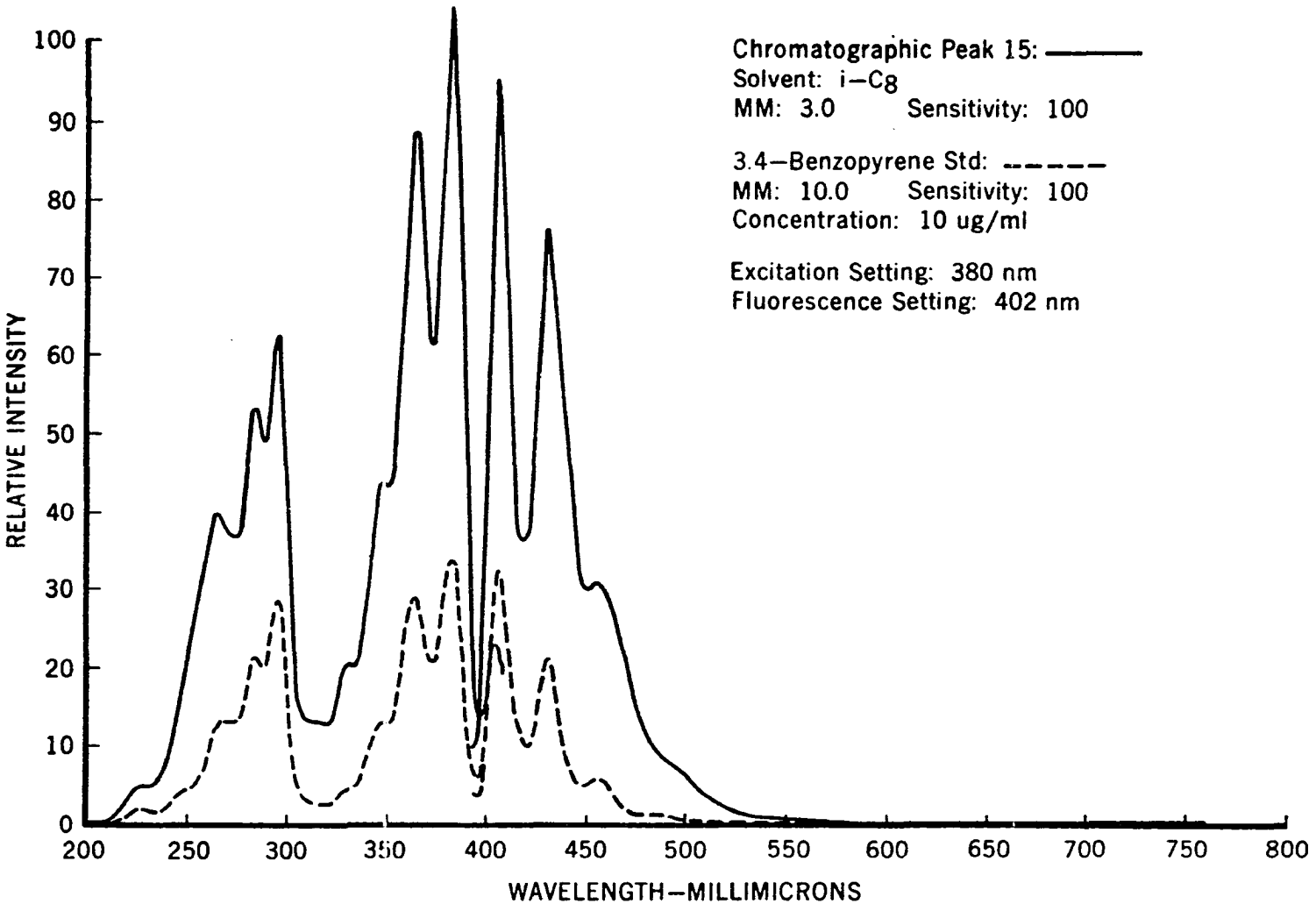

Figure 14.--Excitation and Fluorescence Spectra of Chromatographic Peak 15 from SRF Carbon Black Extract and 3.4–benzopyrene.

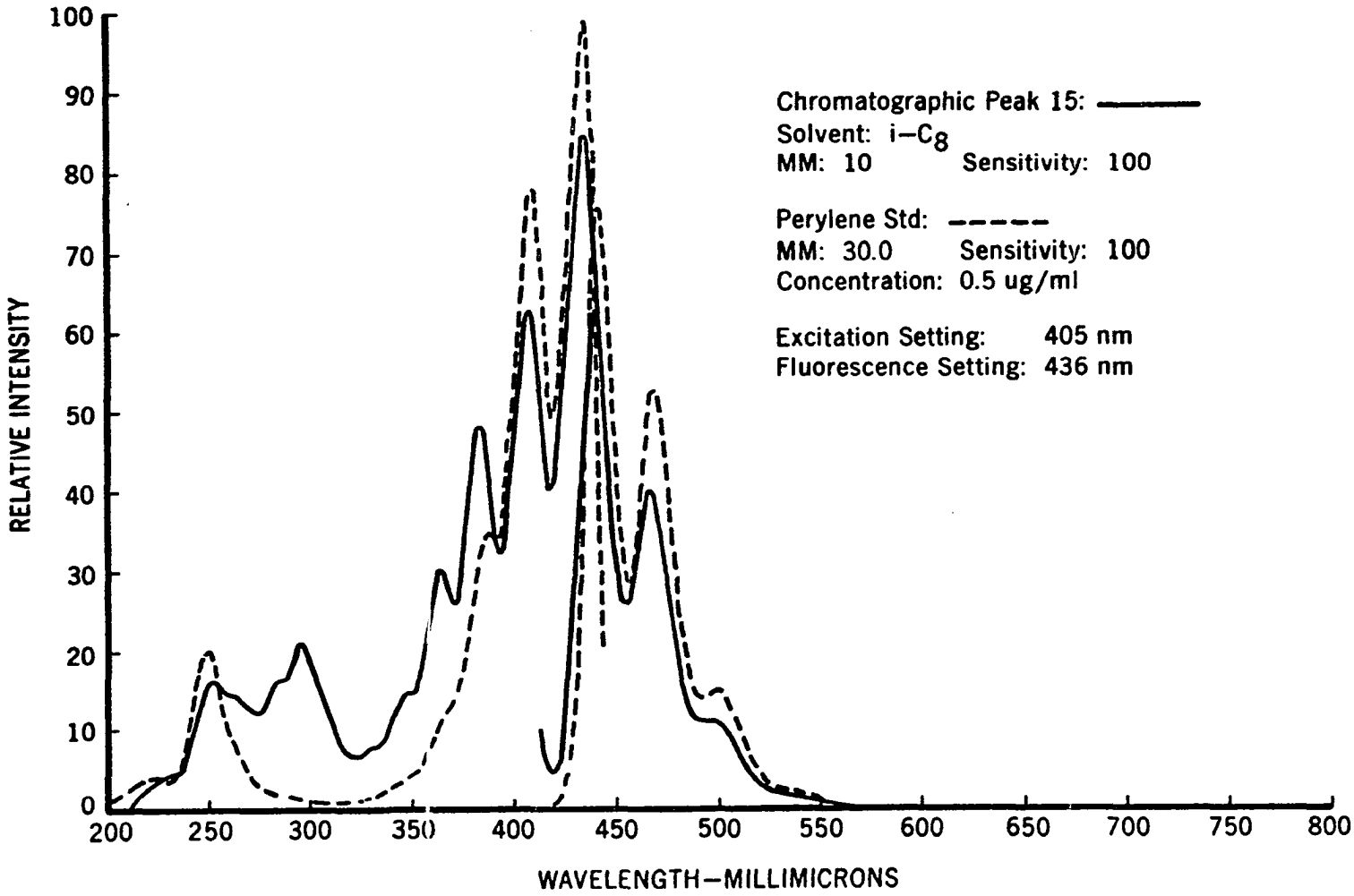

Figure 15.-- Excitation and Fluorescence Spectra of Chromatographic Peak 15 from SRF Carbon Black Extract and Perylene.

spectrum of this peak does not match exactly with that of the std which probably is due to interference by BAP. The presence of BAP, however, could be confirmed from either the Ex or Fl spectrum or both. The Ex peaks at 295, 362.5, and 382 nm and Fl peaks at 405, 430, and 455 nm are sharper than even the std. All these peaks match perfectly with the std and BEP and perylene do not seem to interfere at all. Perylene in peak 15 could be easily identified from its Fl spectrum which compares very well with that of the std. However, there is a little difference in its Ex spectrum as compared to the std in that it has one extra peak at 295 nm which may be due to the presence of BAP.

Gas chromatographic peak 16 was collected during a t_R of 56.0 to 57.36 min. Its identity was established by peak enhancement technique as well as by its Ex and Fl spectra as compared to those of o-phenylene pyrene std (see Appendix C for its SPF spectrum). Todd (66) did not show the presence of this substance in his carbon black extract. However, its presence was shown definitely in soot samples by Chakraborty and Long (95).

Chromatographic peak no 17 was collected during a t_R of 59.0 to 67.0 min. The contents of the collection tube were taken into i-C_8 and this solution was examined for 1.12-benzoperylene and anthanthrene by SPF. Figures 16 and 17 compare the Ex and Fl spectra of peak 17 to those of 1.12-benzoperylene and the anthanthrene std, respectively. It is clear that these two PCAHs do not separate completely under the conditions employed. The identity of anthanthrene was confirmed beyond any doubts from its Ex and Fl spectra. However, the presence of 1.12-benzoperylene in peak 17 could be confirmed from the Ex spectrum. Its Fl

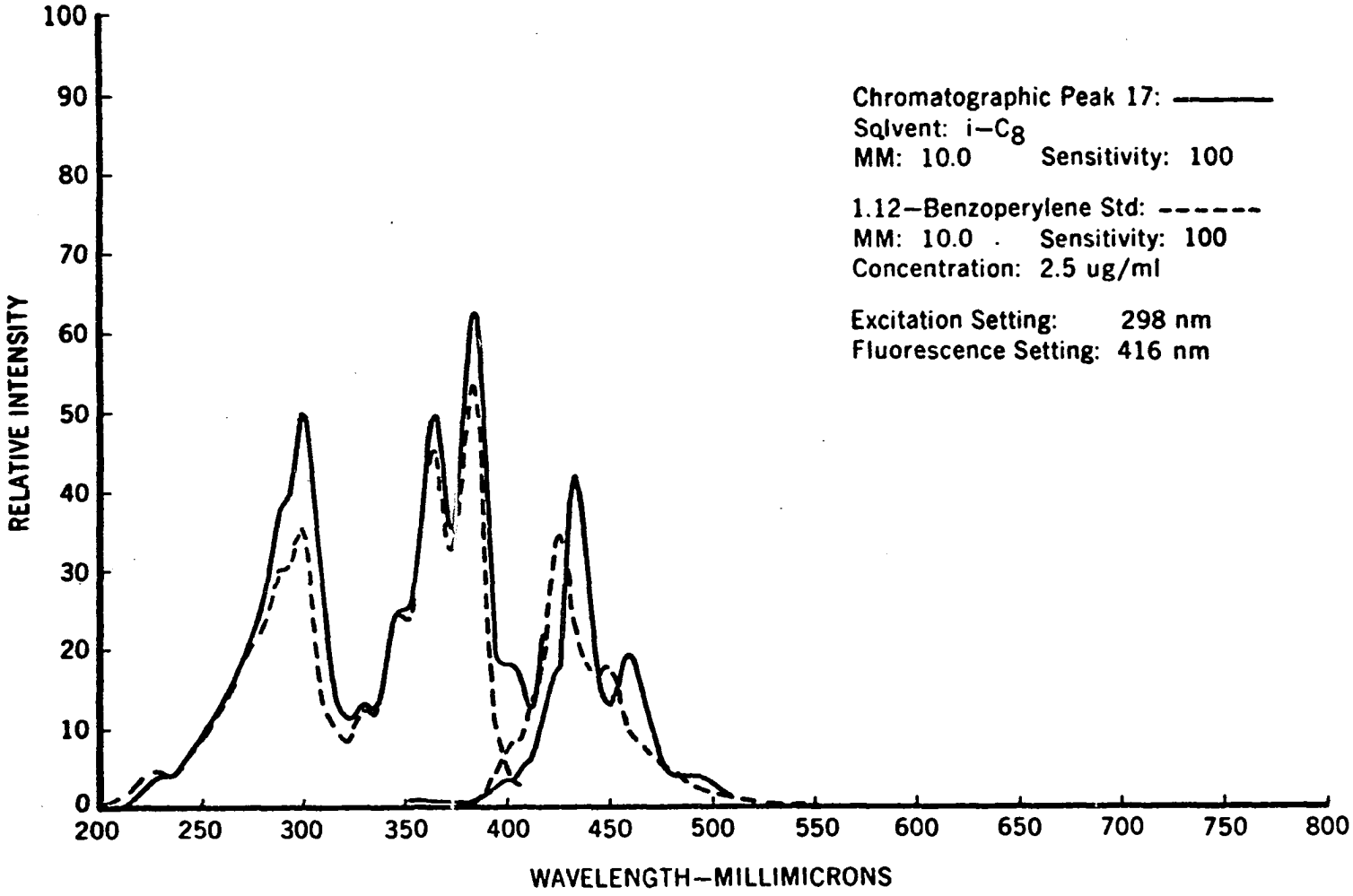

Figure 15.-- Excitation and Fluorescence Spectra of Chromatographic Peak 17 from SRF Carbon Black Extract and 1.12–benzoperylene.

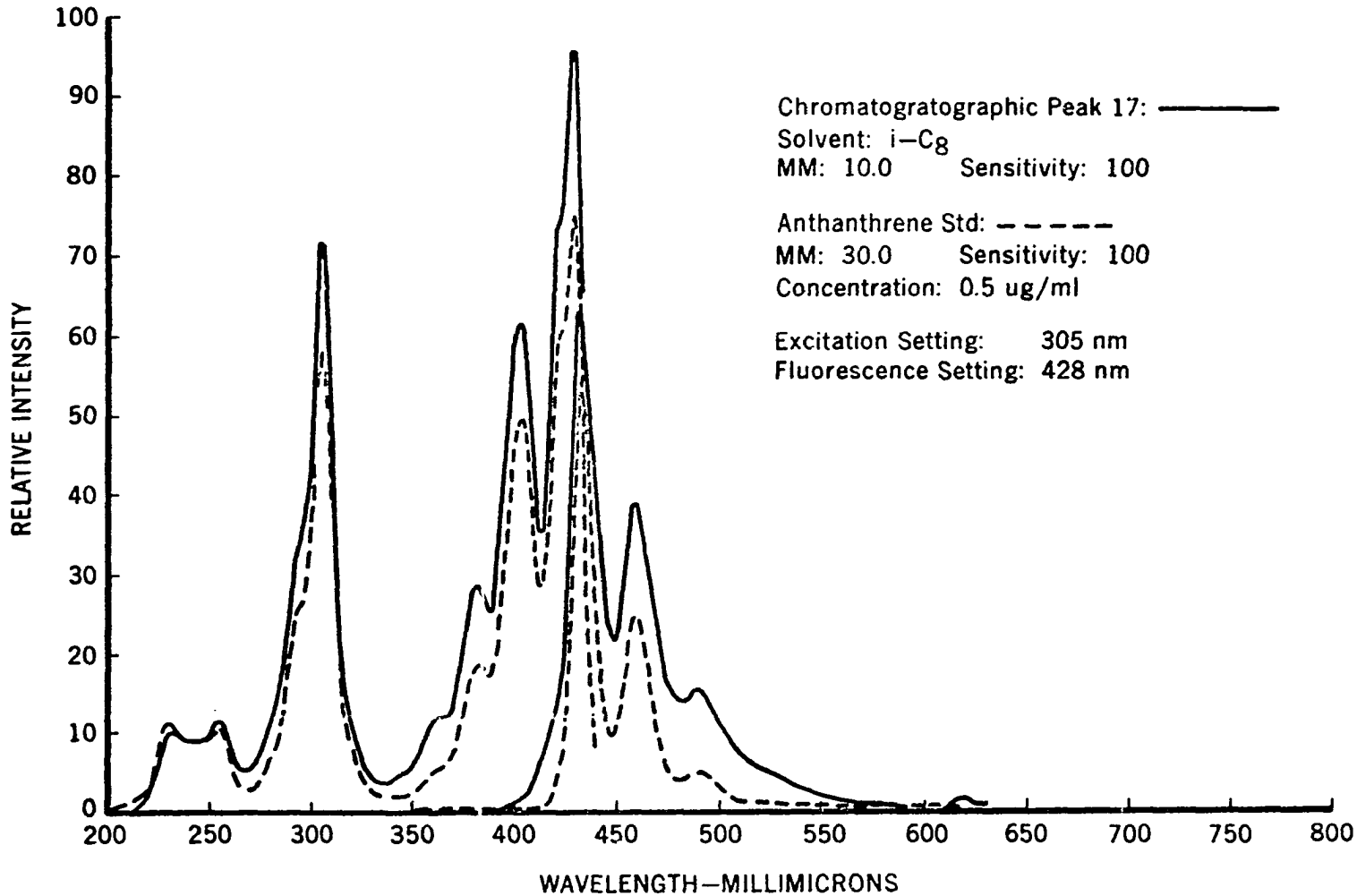

Figure 17.-- Excitation and Fluorescence Spectra of Chromatographic Peak 17 from SRF Carbon Black Extract and Anthanthrene.

spectrum had shifted to the left by about 1.5 nm which could be due to the presence of anthanthrene. The presence of these two PCAHs has been confirmed also in "furnace thermal" black by Todd (66) and in soot samples by Chakraborty and Long (94).

The identity of peak 18 was established by its long t_R and also by the enhancement of peak technique. It was found to be coronene. Its presence was further confirmed by the Ex and Fl spectra compared with that of the std. The spectra of peak 18 along with those of the coronene std are shown in Appendix C.

Gas chromatographic peak no 19 and 20 were also collected and their Ex and Fl spectra were recorded as shown in Appendix C. No definite conclusions as to their identity were made. However, the Ex and Fl spectra of peak 19 were compared with those of 3.4,8.9- and 3.4,9.10-dibenzpyrene. The Fl spectrum of peak 19 was shifted to the right by about one nanometer from the corresponding spectrum of 3.4,9.10-dibenzpyrene std. In its Ex spectrum, the peaks appearing at 335, 360, and 372 nm were also present in the spectrum of 3.4,9.10-dibenzpyrene std. It could probably be 3.4,9.10-dibenzpyrene contaminated with some other compound. No mention was made about the presence of these PCAHs by Todd (66).

Quantitation of PCAHs in SRF Carbon Black Extract

The estimation of the PCAHs would have been easier and simpler if all the peaks in the gas chromatogram had only one component. In other words, if a suitable chromatographic column could be found which is capable of resolving each and every PCAH into a separate peak, then

the estimation of each PCAH in the sample chromatogram could be done from a plot of peak area against concentration of the corresponding compound. Unfortunately, the column used in this investigation, as well as those used in the previous studies, was not able to resolve all the PCAHs completely. Therefore, the PCAHs of the chromatographic peaks that have only one component such as 6, 7, 14, 16, and 18, were estimated from the std plots of peak area against concentration of the corresponding PCAHs. Figure 18 shows a std plot for pyrene. Standard plots for fluoranthene, 9,10-dimethyl-1.2-benzanthracene, o-phenylene pyrene, coronene, and BAP are given in Appendix D.

Estimations of the PCAHs in the remaining identified peaks 4, 11, 15, and 17 were done by SPF analyses. Spectrophotofluorometry offers very high sensitivity, as well as high specificity. The very high specificity of this technique has been utilized by Sawicki et al. (106) in the identification of PCAHs such as BAP, BEP, perylene, and anthanthrene from a mixture containing 40 to 50 PCAHs. The same technique was utilized in the present study for the quantitation of the PCAHs in the chromatographic peaks 4, 11, 15, and 17. The quantity of each PCAH identified in the carbon black extract by either of the two methods described above is given in Table 10. Although peak 10 was identified, it could not be estimated because no authentic std was available at that time.

Snyder (63) found that from time to time the lamp in the SPF will strike its arc to the envelope of the lamp instead of across the electrodes. It seems necessary to watch the lamp as it strikes an arc and to turn it off immediately and try again if the arc does not light

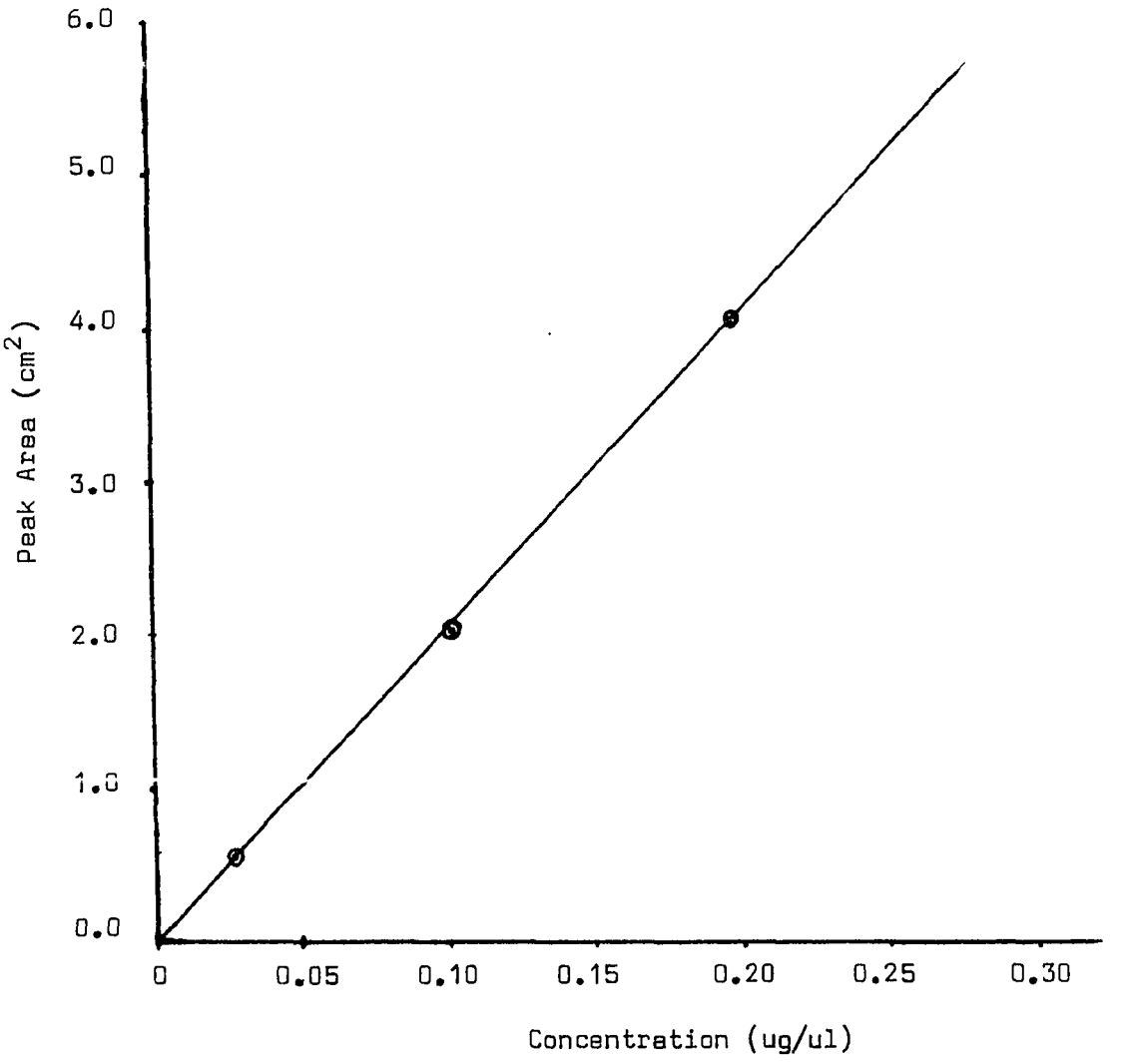

Figure 18.--Plot of concentration against peak area (at 200°C) for pyrene.

TABLE 10

POLYCYCLIC AROMATIC HYDROCARBONS IDENTIFIED AND THEIR AMOUNTS CALCULATED IN SRF CARBON BLACK

Peak No	Compound Identified	Quantitation (PPM) by SPF Method	Peak Area/Graphical Method
4	Anthracene	1.22	-
	Phenanthrene	4.50	-
6	Fluoranthene	6.50	6.68
7	Pyrene	45.8	45.92
10	Benzo(mno)fluoranthene	-	-
11	1.2-Benzanthracene	0.82	-
	Chrysene	3.19	-
14	9,10-Dimethyl-1.2-benzanthracene	1.58	1.50
15	Benzo(e)pyrene	10.48	-
	Benzo(a)pyrene	6.54	-
	Perylene	0.86	-
16	o-Phenylene pyrene	7.81	7.42
17	1.12-Benzoperylene	38.90	-
	Anthanthrene	149.42	-
18	Coronene	-	94.60

satisfactorily in order to avoid damage to the lamp. Also, the lamp output varies from day to day because the arc rarely strikes in exactly the same place again. This problem can be compensated by running a Std with each determination at the same time as is seen in the Figures 8, 9, 11, 12, 13, 14, 15, 16, and 17. Moreover, the estimation of a PCAH was averaged from at least three peaks of the Ex and Fl spectra of that PCAH. This method is rapid and reliable. It was possible for the author to do 4 to 5 determinations per one work day.

CHAPTER V

SUMMARY AND CONCLUSIONS

Polycyclic aromatic hydrocarbons may be formed in any combustion process involving fossil fuels or simply components of carbon and hydrogen. There is a clear evidence that airborne PCAHs found in occupational environments, especially in relation to the products of burning, refining, and distilling of fossil fuels, are responsible for specific adverse biologic effects in man. These effects include cancer of the skin and lungs, non-allergic contact dermatitis, photosensitization reactions, hyperpigmentation of the skin, folliculitis, and acne (37). However, there is no direct evidence that in the concentrations of PCAHs found in the urban or non-urban air PCAHs cause any of these skin diseases. Similarly, no direct causal relationship has been established between any particular PCAH and lung cancer. Nevertheless, it is a well-known fact that the incidence of lung cancer among urban dwellers is twice that of those living in rural areas; and within urban communities, the incidence is even higher near industrial areas where fossil-fuel products are concentrated in the air (37). The foregoing fact clearly points out a definite relationship between the extent of air pollution and the incidence of lung cancer.

Carbon black is a product of incomplete combustion of fossil

fuels (such as natural gas, oil residue, and heavy aromatic tar). During the last quarter of this century, the presence of some PCAHs has been well established in carbon black, synthetic rubber, and rubber products. Some of these PCAHs from carbon black and rubber products have been shown to be carcinogenic. A few of the carcinogenic PCAHs have also been found to be present in the urban air.

There has been a great deal of research on the analysis of these PCAHs. Most of the analytic techniques developed thus far are either suitable for individual hydrocarbons or else are quite tedious and very time consuming. Also most of these are of a qualitative nature. This investigation was conceived because the Department of Environmental Health of the University of Oklahoma Health Sciences Center is involved in research with carbon black and rubber dust, and because of carbon black being a potential public health hazard.

The identity of the PCAHs in a semi re-inforcing furnace carbon black was established by the following procedure which involved: a) Soxhlet extraction of a weighed amount of carbon black in benzene - the extract then being transferred quantitatively into CS_2; b) the development of gas chromatogram on the 5' x 0.25", OD, 3 per cent OV-1, stainless steel column, and comparison of retention times with those of standards PCAHs; c) enhancement of each separated peak by a PCAH standard; and d) collection of separated peaks, examining the collected peaks in i-C_8 with the SPF and the Ex and Fl spectra compared to those of standard solutions.

The following PCAHs from the carbon black extract were identified as described in the above procedure:

a) Phenanthrene

b) Anthracene

c) Fluoranthene

d) Pyrene

e) Chrysene

f) 1.2-Benzanthracene

g) Benzo(mno)fluoranthene identified by comparison of UV spectra of the collected peak with that reported in the literature (30)

h) 9,10-Dimethyl-1.2-benzanthracene

i) Benzo(e)pyrene

j) Benzo(a)pyrene

k) Perylene

l) O-phenylene pyrene

m) 1.12-Benzoperylene

n) Anthanthrene

o) Coronene

The estimation of the PCAH was performed by two different procedures: a) by calculating the peak area of each separated peak and then reading the concentration directly from the plot of concentration against the peak area of the corresponding PCAH standard; and b) by recording the Ex and Fl spectra of the sample peak along with those of a quantitative PCAH standard solution (and then by comparison of peak height under similar conditions). Thus, fluoranthene, pyrene, 9,10-dimethyl-1.2-benzanthracene, O-phenylene pyrene, and coronene were estimated by the first method and the remaining PCAHs were estimated by the second method. There appears to be a good agreement between the two methods.

Based on the evidence presented and the techniques developed in this investigation, the following conclusions have been drawn:

a) The technique developed is sensitive, accurate, rapid and suitable for the analysis of polycyclic aromatic hydrocarbons.

b) The results of the procedure are reproducible under the same conditions and the procedure is capable of detecting a larger number of PCAHs than any other method.

c) The compounds in chromotographic peaks can be collected and confirmed by SPF and UV absorption analysis.

d) The procedure is adaptable for other types of samples, including air particulates and dust samples.

Further improvements in this technique are possible. A few improvements are suggested here:

a) The use of the most recent version of the flame ionization detector which has a sample sensing capability of 10^{-10}g or the use of an electron capture detector for increased sensitivity.

b) The selection of a more suitable column which may completely resolve the PCAHs present in the sample.

c) The use of newly developed liquid stationary phases which can stand higher column temperatures and may under suitable conditions separate relatively non-volatile polycyclic aromatic hydrocarbons.

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APPENDIX A

Abbreviations

ABBREVIATIONS

A list of abbreviations used in this text:

Abbreviation	Name
DMBA	9,10-dimethyl-1.2-benzanthracene
BEP	1.2-benzopyrene (benzo[e]pyrene)
BAP	3.4-benzopyrene (benzo[a]pyrene)
F	fluoranthene
i-C_8	isooctane
CS_2	carbon disulfide
PCAHs	polycyclic aromatic hydrocarbons
UV	ultraviolet
GC	gas chromatography
GLC	gas liquid chromatography
SPF	spectrophotofluorometer
TLC	thin-layer chromatography
lbs/in^2	pounds per square inch
cm	centimeter
OD	outer diameter
ID	internal diameter
Kg	kilogram
ft or '	foot
in or "	inch
nm	nanometer
std	standard

ABBREVIATIONS--Continued

Abbreviation	Name
Bz	benzene
ml	milliliter
ul	microliter
ug	microgram
t_R	retention time
FI	flame ionization
TC	thermal conductivity
Ex	excitation
Fl	fluorescence
MM	meter multiplier
SS	stainless steel
mm	millimeter

APPENDIX B

Definitions

DEFINITIONS

List of definitions of the terms used in this text:

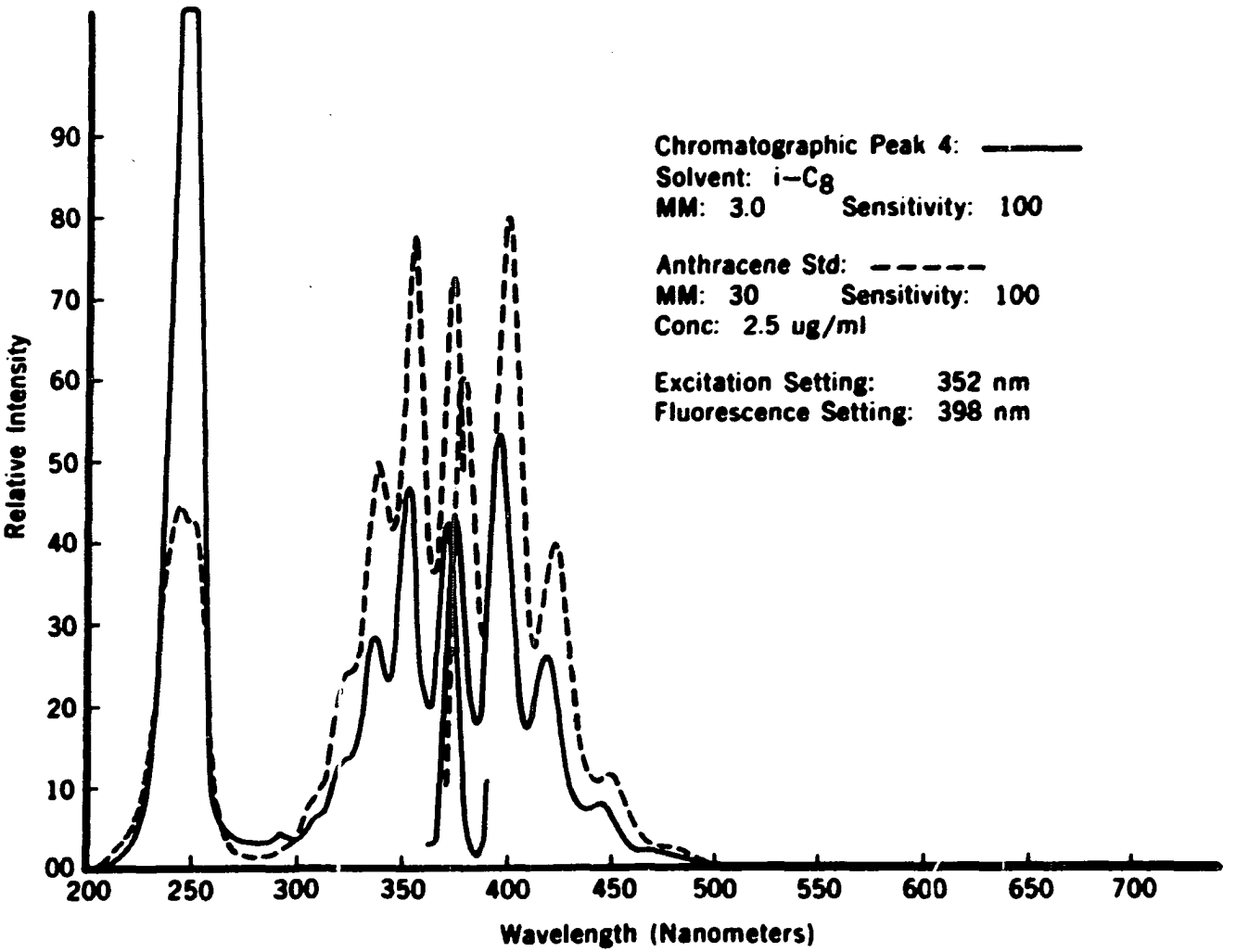
Terms	Definition
Cancer	an atypical, undifferentiated, cellular growth which proliferates progressively and which possesses a total or far-reaching autonomy as a biologic phenomena from the host organism.
Carcinogens	chemical, physical, and parasitic agents of natural and man-made origin which are capable, under proper conditions of exposure, of producing cancer in animals, including man, in one or several organs and tissues, regardless of the route of exposure and the dose and physical state of the agent used.
Co-carcinogenesis	a process whereby cancer is induced in animal or man by the combined action of two or more agents, either by a single exposure or, (as is more common in both the laboratory and the environment) by repeated exposures.
Anti-carcinogenesis	a process whereby cancer is inhibited in animal or man by the action of an agent when administered in single or multiple doses before, during, or after treatment with a carcinogen.
Initiation	an irreversible process specific for carcinogens, which transforms normal cells into dormant tumor cells, a process similar to mutation.
Promotion	a process in which a single or multiple application of an agent leads the dormant tumor cells to division and multiplication.
Luminescence	a phenomena in which dissipation of energy takes place by re-emission of radiation in random directions.
Fluorescence	involves the immediate release of absorbed light (ultraviolet or visible) energy from an atom or a molecule.

DEFINITIONS--Continued

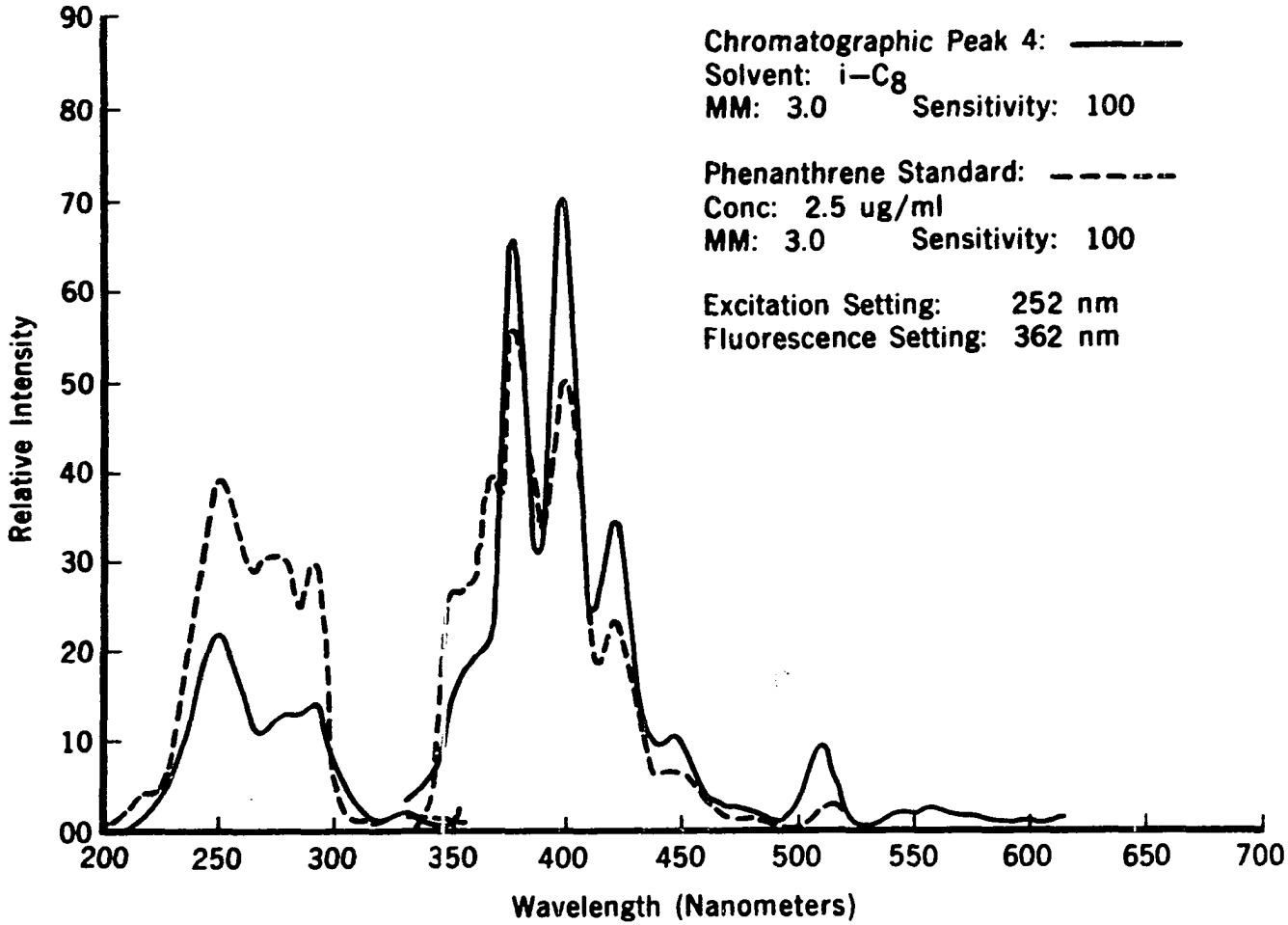
Terms	Definition
Phosphorescence	involves the delayed release of absorbed light energy from an atom or a molecule.
R_f	R_f is defined as the ratio of the distance traveled by the compound to the distance traveled by the solvent.
t_R	retention time may be defined as the time along the baseline from the injection point to the perpendicular line dropped from the top of an eluted peak of a given compound.

APPENDIX C

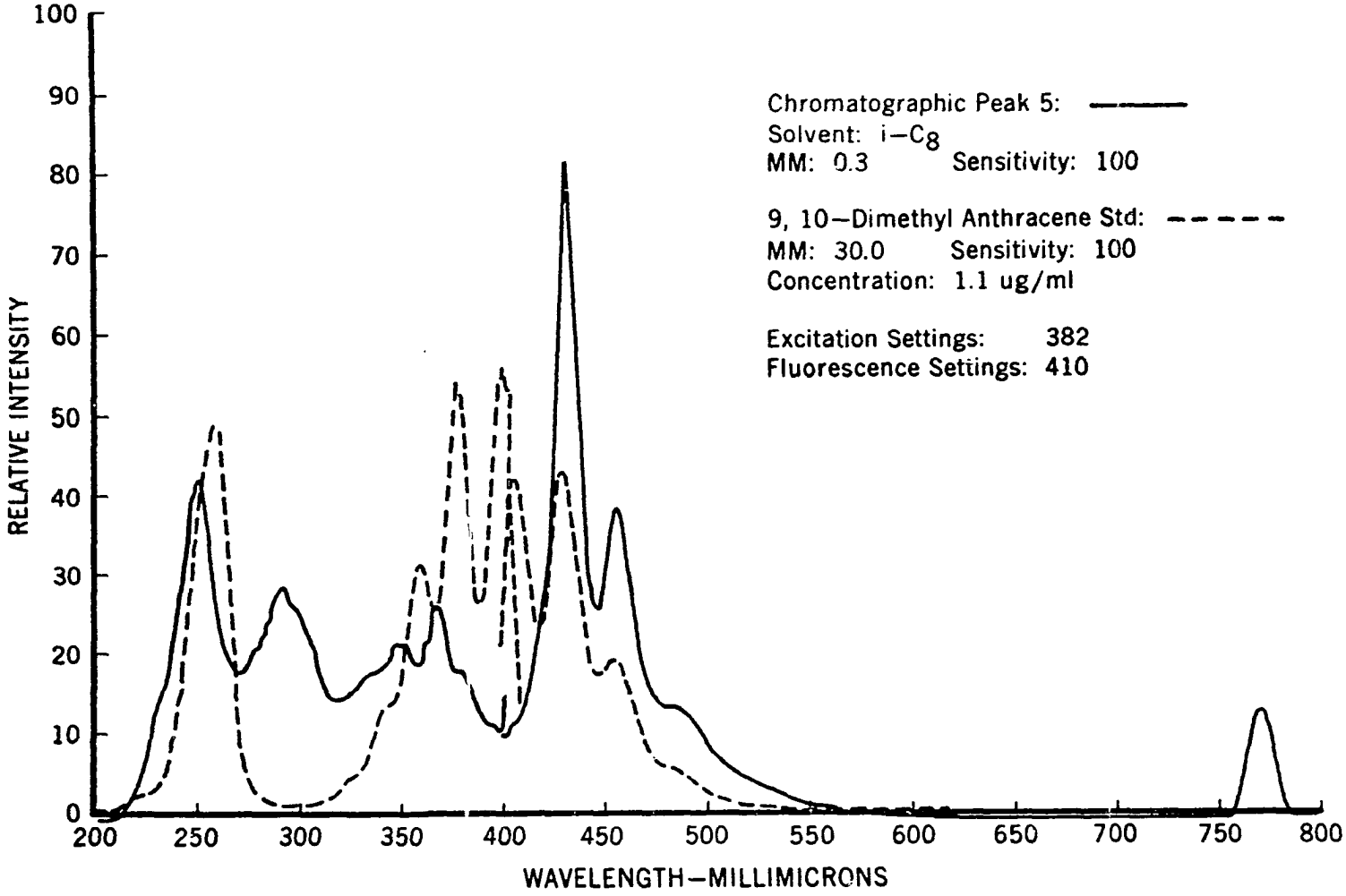
Excitation and Fluorescence Spectra of Chromatographic Peaks 4, 6, 7, 8&9, 11, 14, 15, 16, 17, 18, and 19, along with Corresponding Standard PCAHs



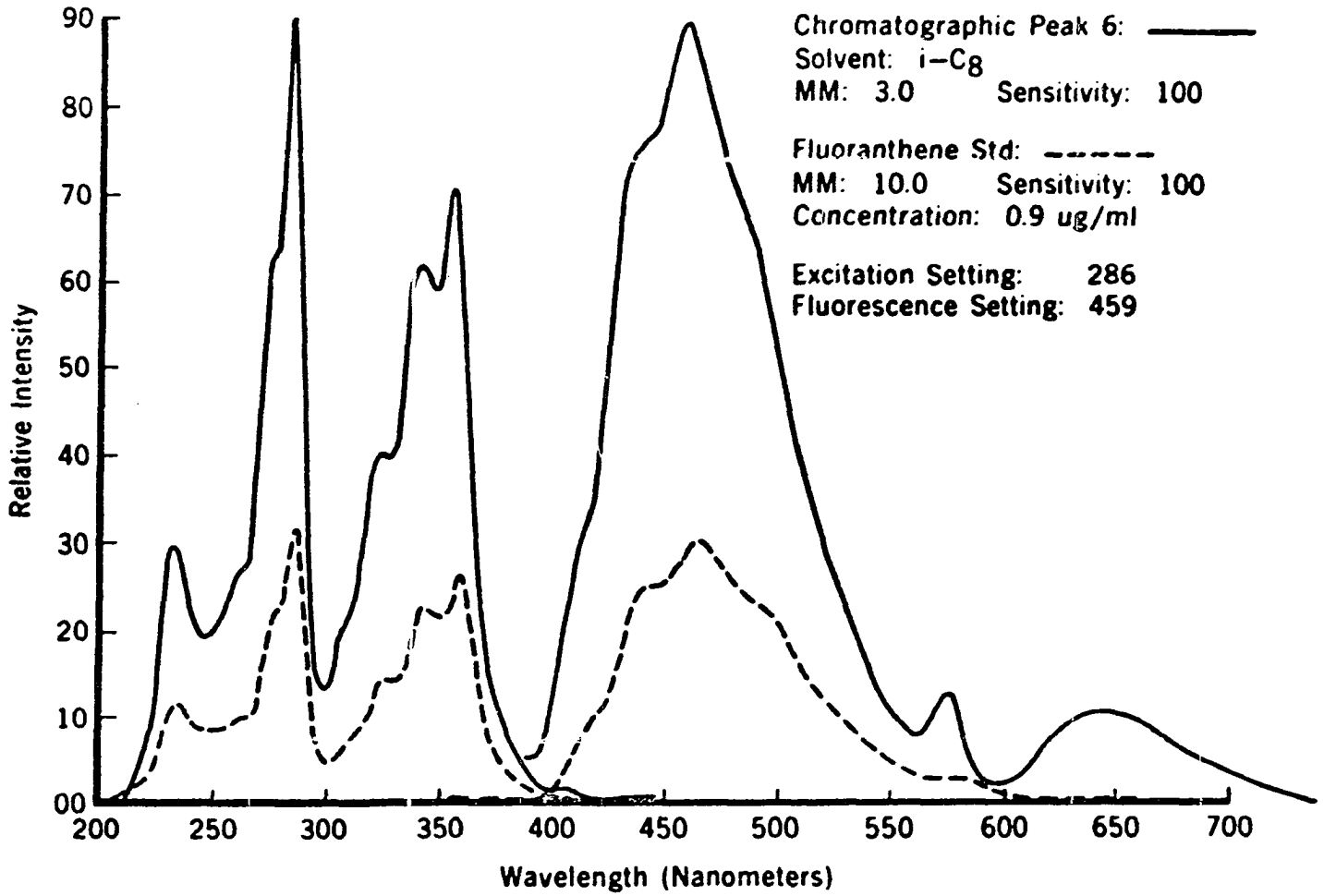
Excitation & Fluorescence Spectra of Chromatographic Peak 4 from Carbon Black Extract and Anthracene in i-C_8.



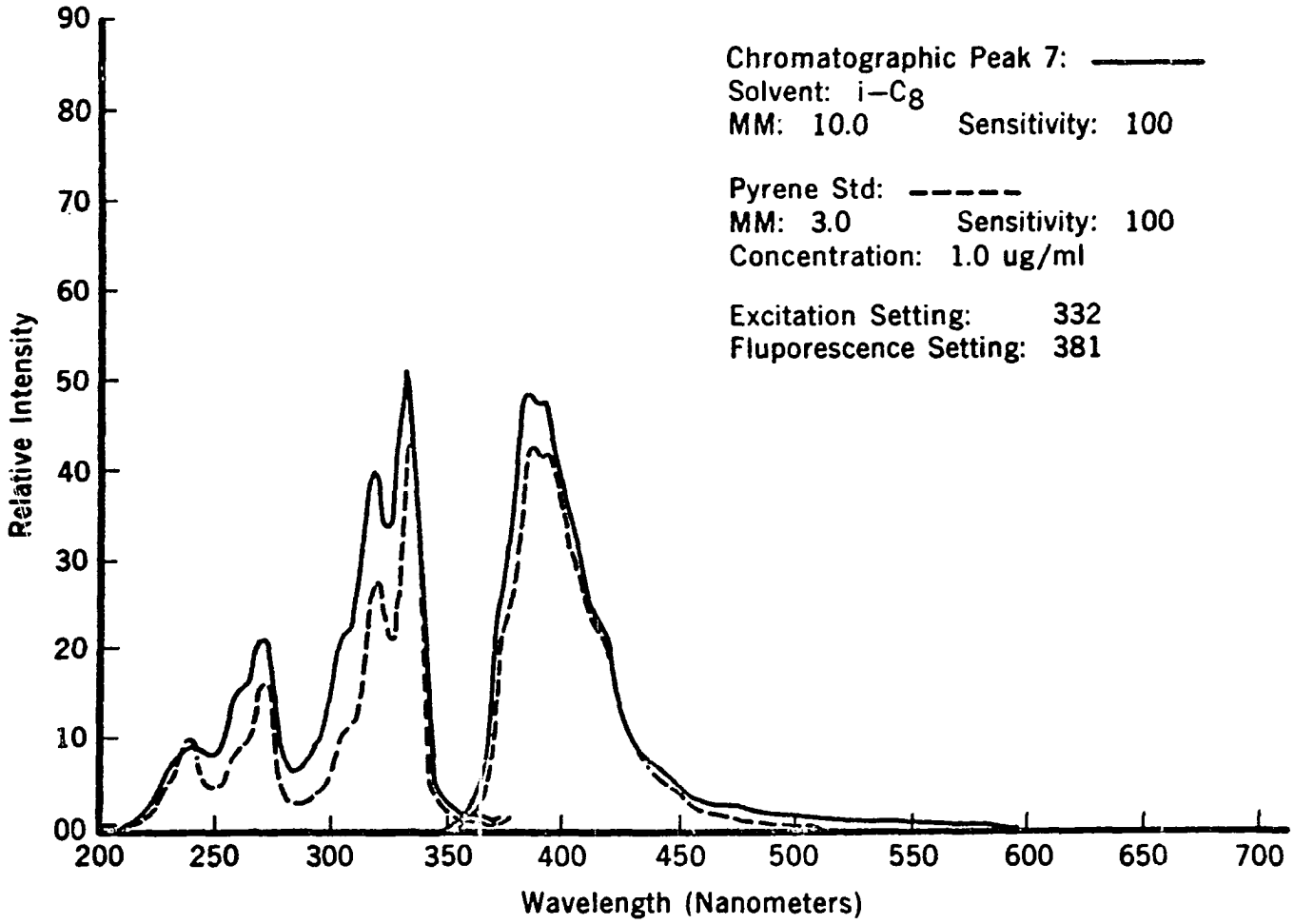
Excitation & Fluorescence Spectra of Chromatographic Peak 4 and Phenanthrene in i-C_8.



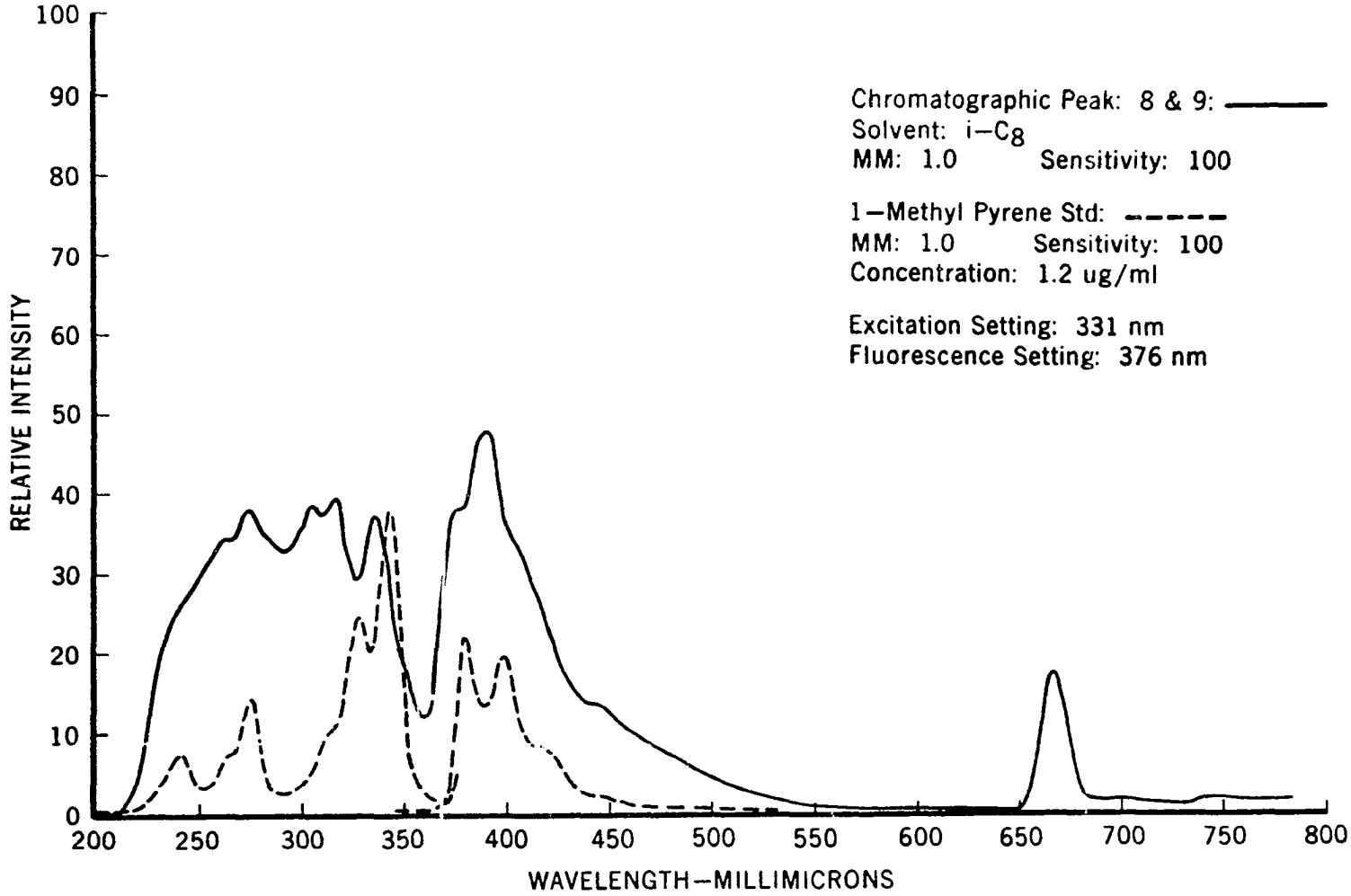
Excitation and Fluorescence Spectra of Chromatographic Peaks 5 from SRF Carbon Black Extract and 9, 10–dimethyl Anthracene.



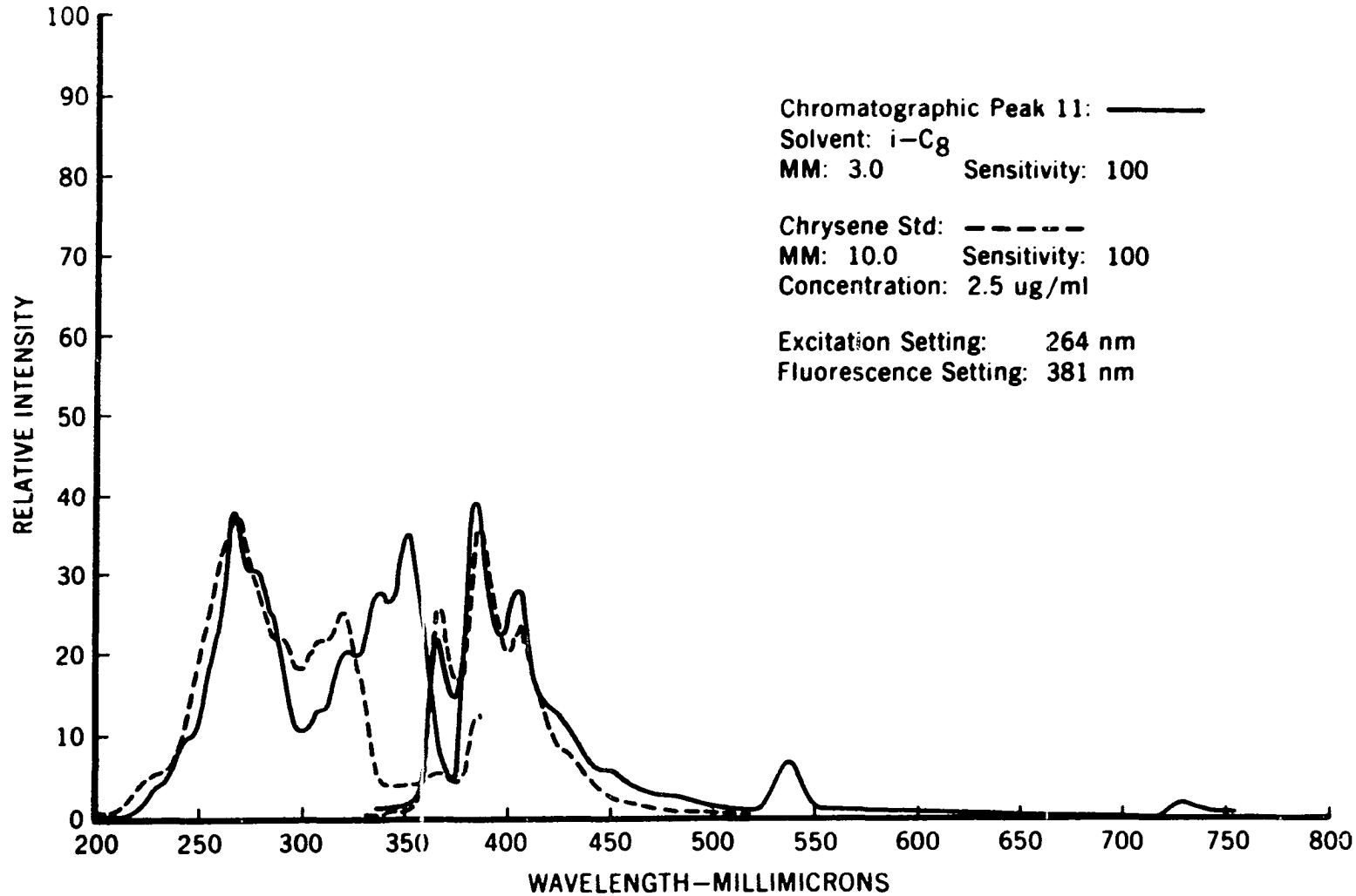
Excitation and Fluorescence Spectra of Chromatographic Peak 6 from SRF Carbon Black Extract and Fluoranthene.



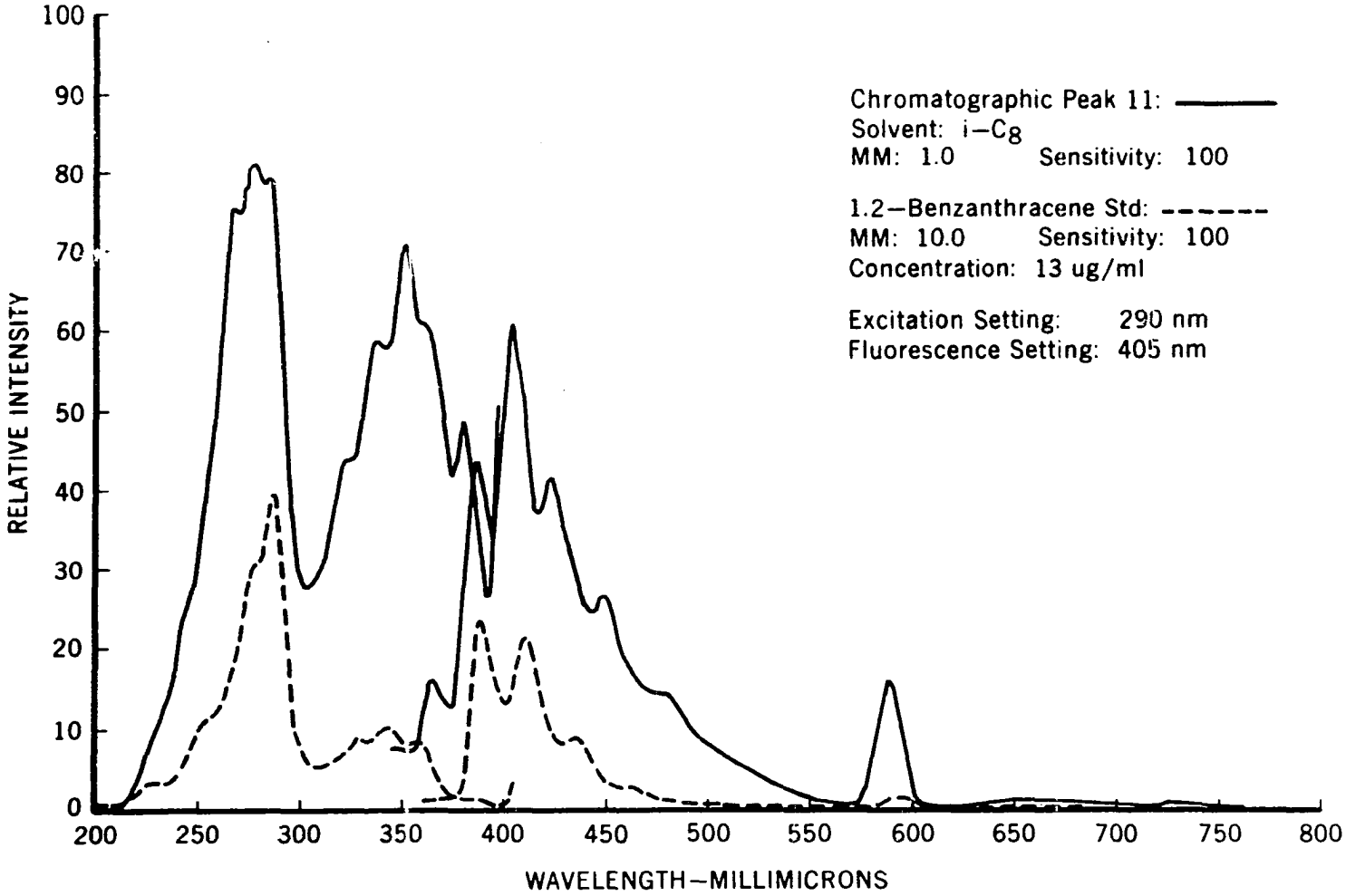
Excitation & Fluorescence Spectra of Chromatographic Peak 7 from SRF Carbon Black and Pyrene.



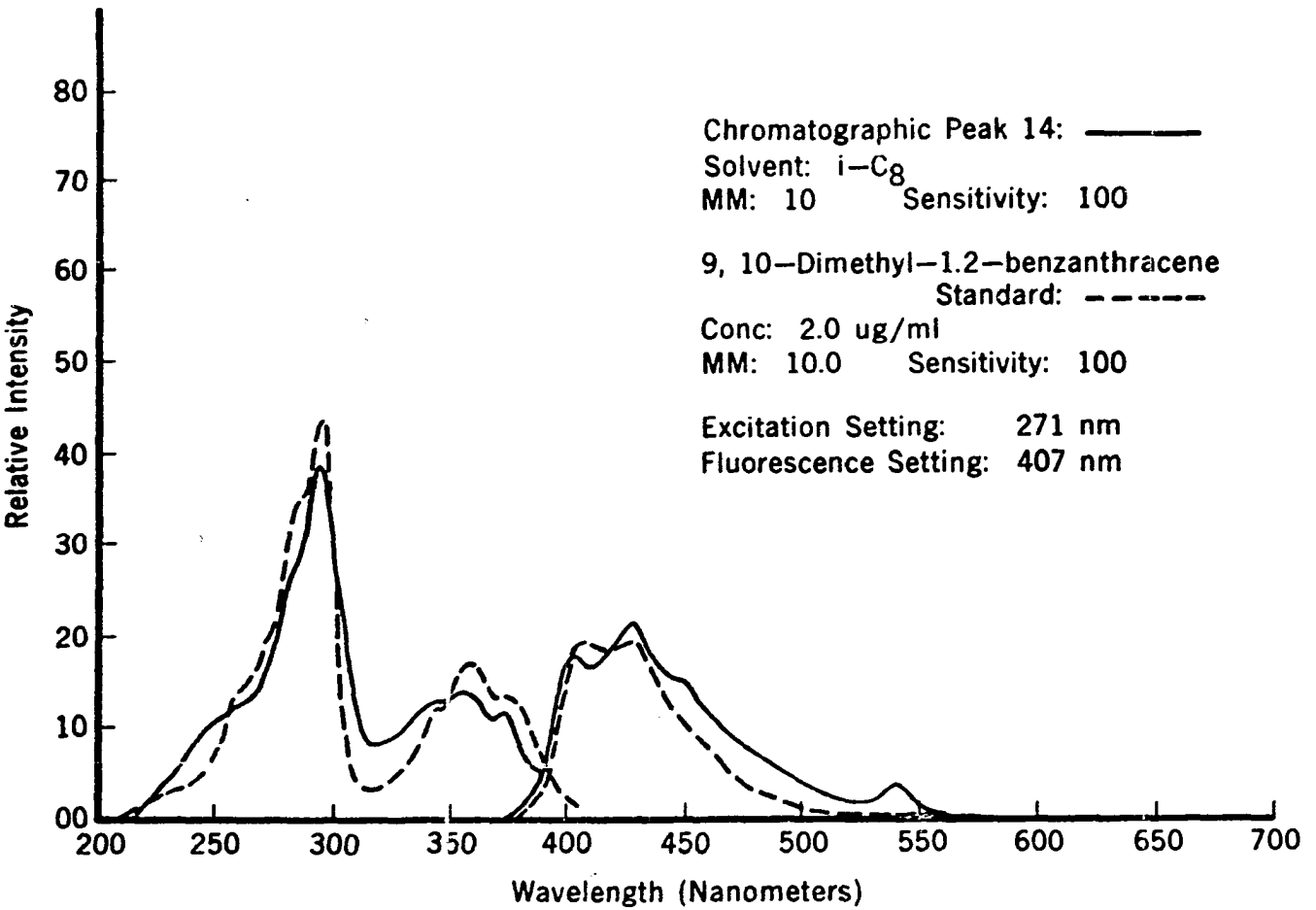
Excitation and Fluorescence Spectra of Chromatographic Peak 8 & 9 from SRF Carbon Black Extract and 1–Methyl Pyrene.



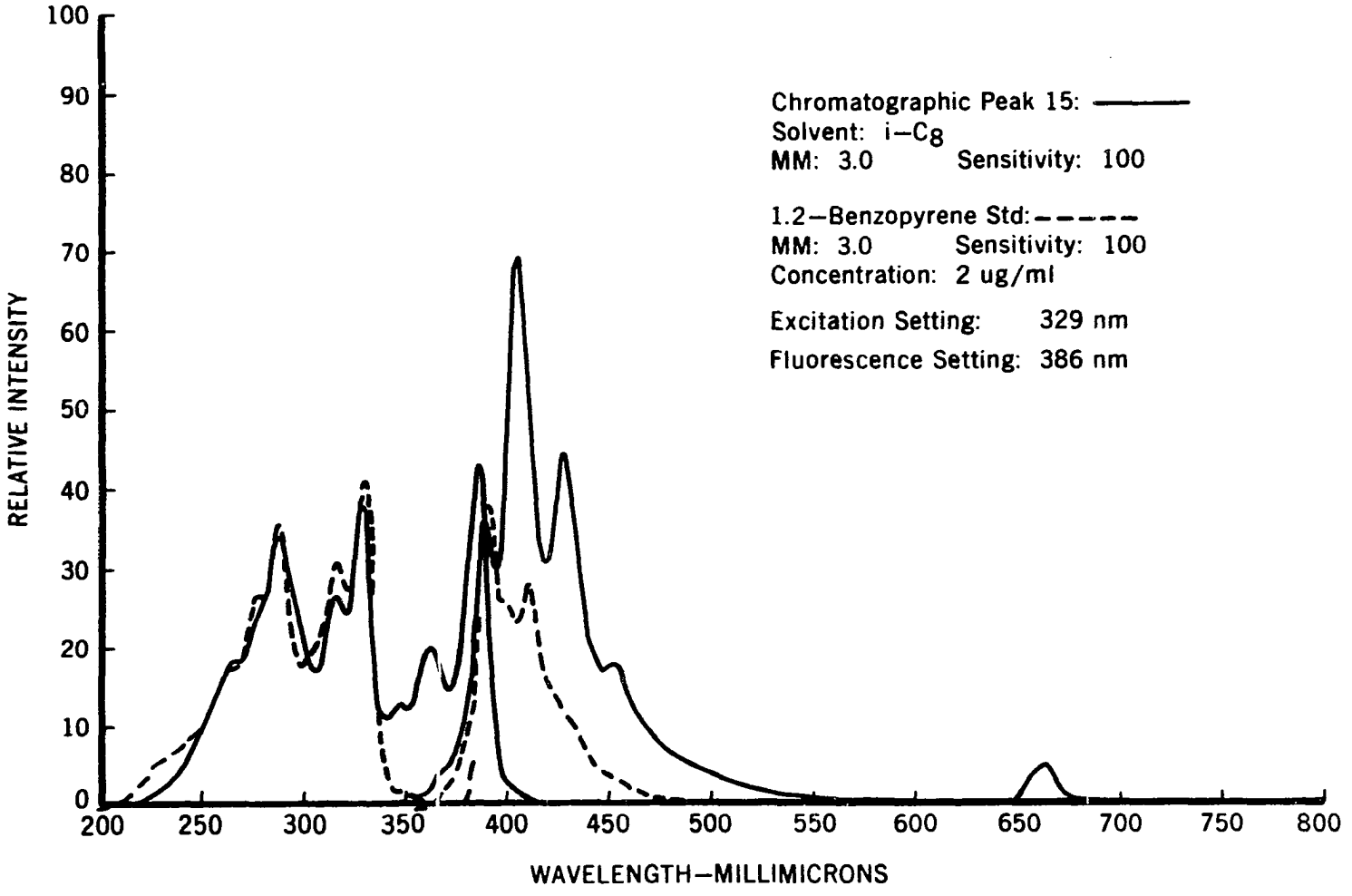
Excitation and Fluorescence Spectra of Chromatographic Peak 11 from SRF Carbon Black Extract and Chrysene.



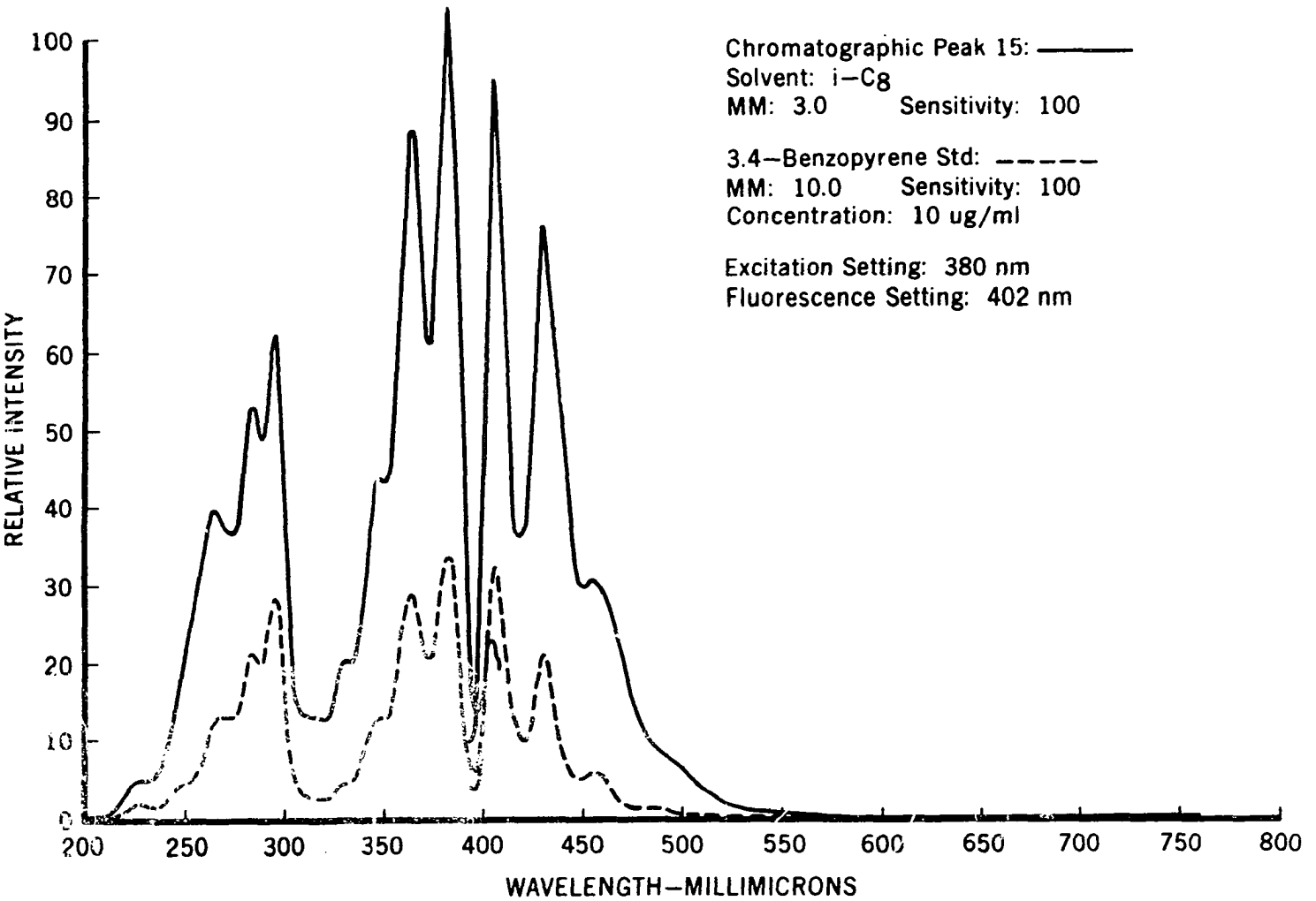
Excitation and Fluorescence Spectra of Chromatographic Peak 11 from SRF Carbon Black Extract and 1.2–benzanthracene.



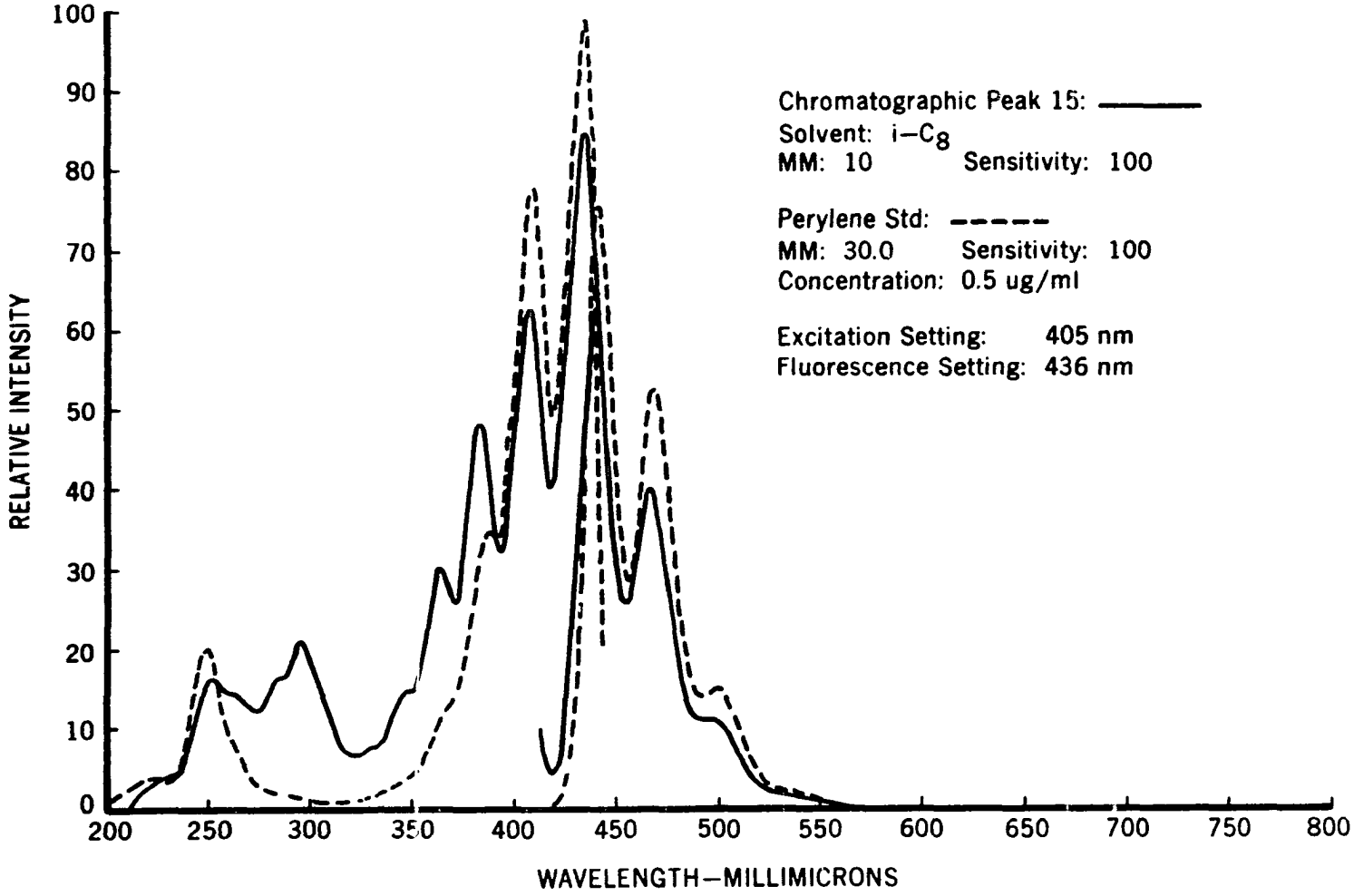
Excitation & Fluorescence Spectra of Chromatographic Peak 14 from Carbon Black and 9, 10-Dimethyl-1.2-benzanthracene.



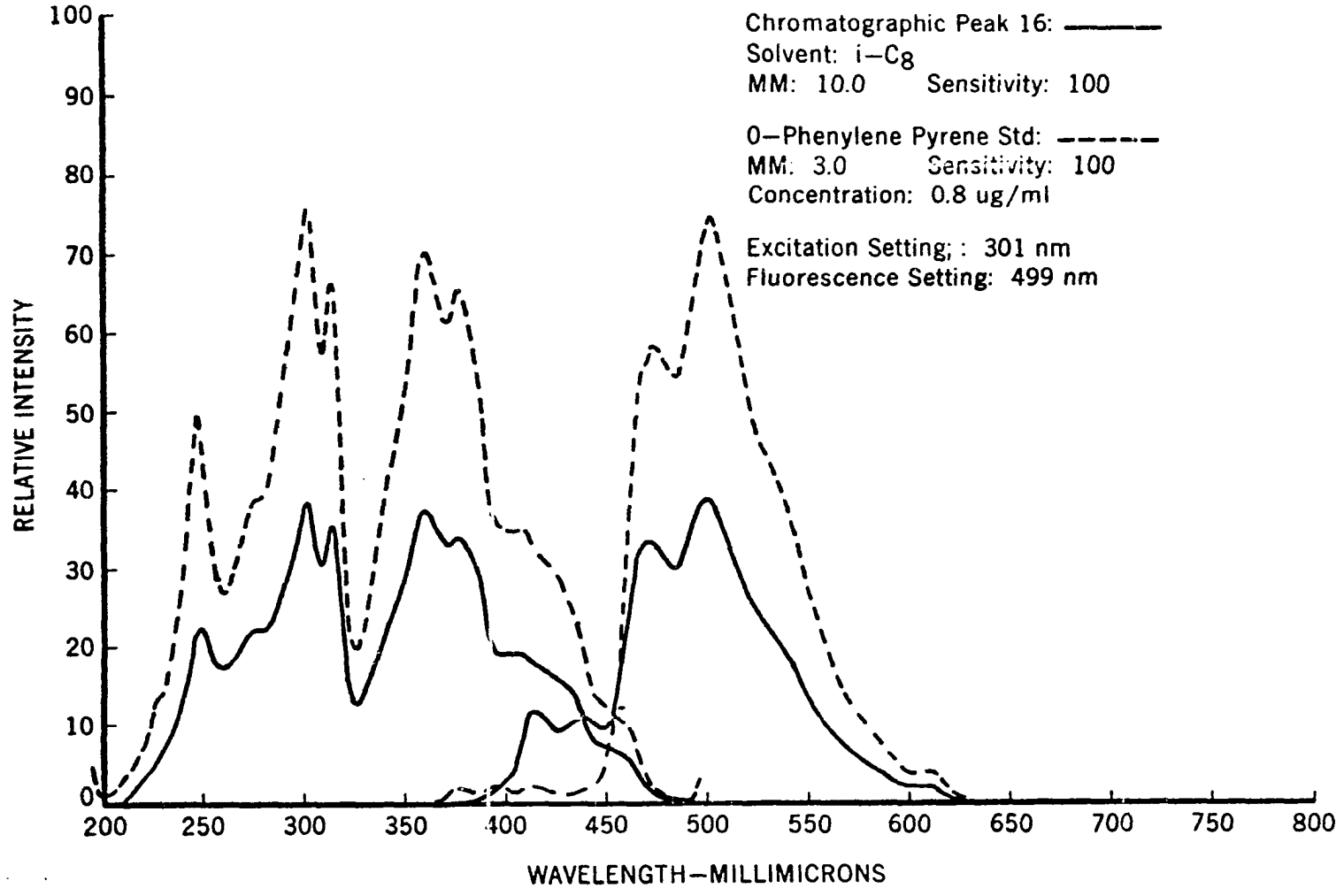
Excitation and Fluorescence Spectra of Chromatographic Peak 15 from SRF Carbon Black Extract and 1.2–benzopyrene.



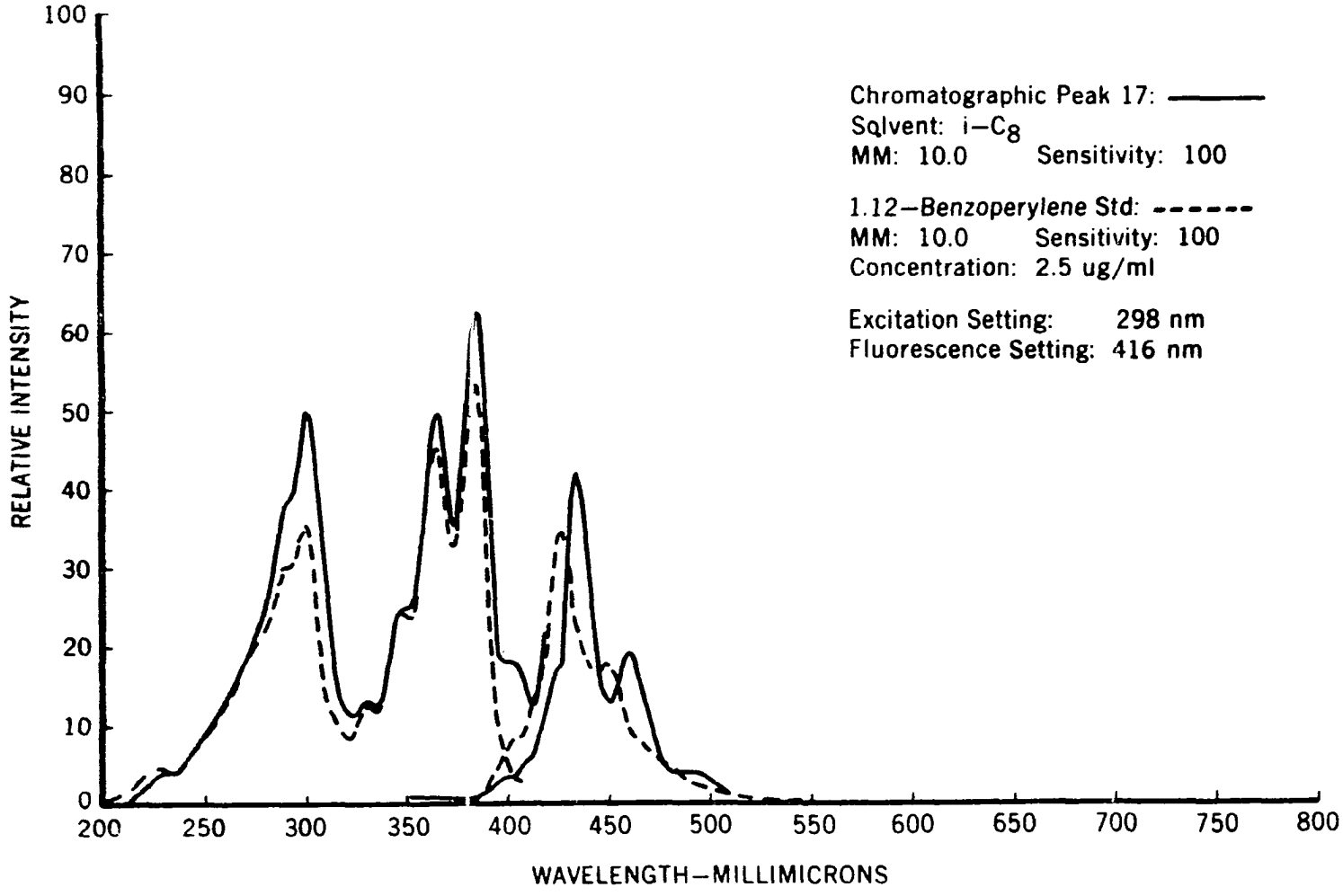
Excitation and Fluorescence Spectra of Chromatographic Peak 15 from SRF Carbon Black Extract and 3.4–benzopyrene.



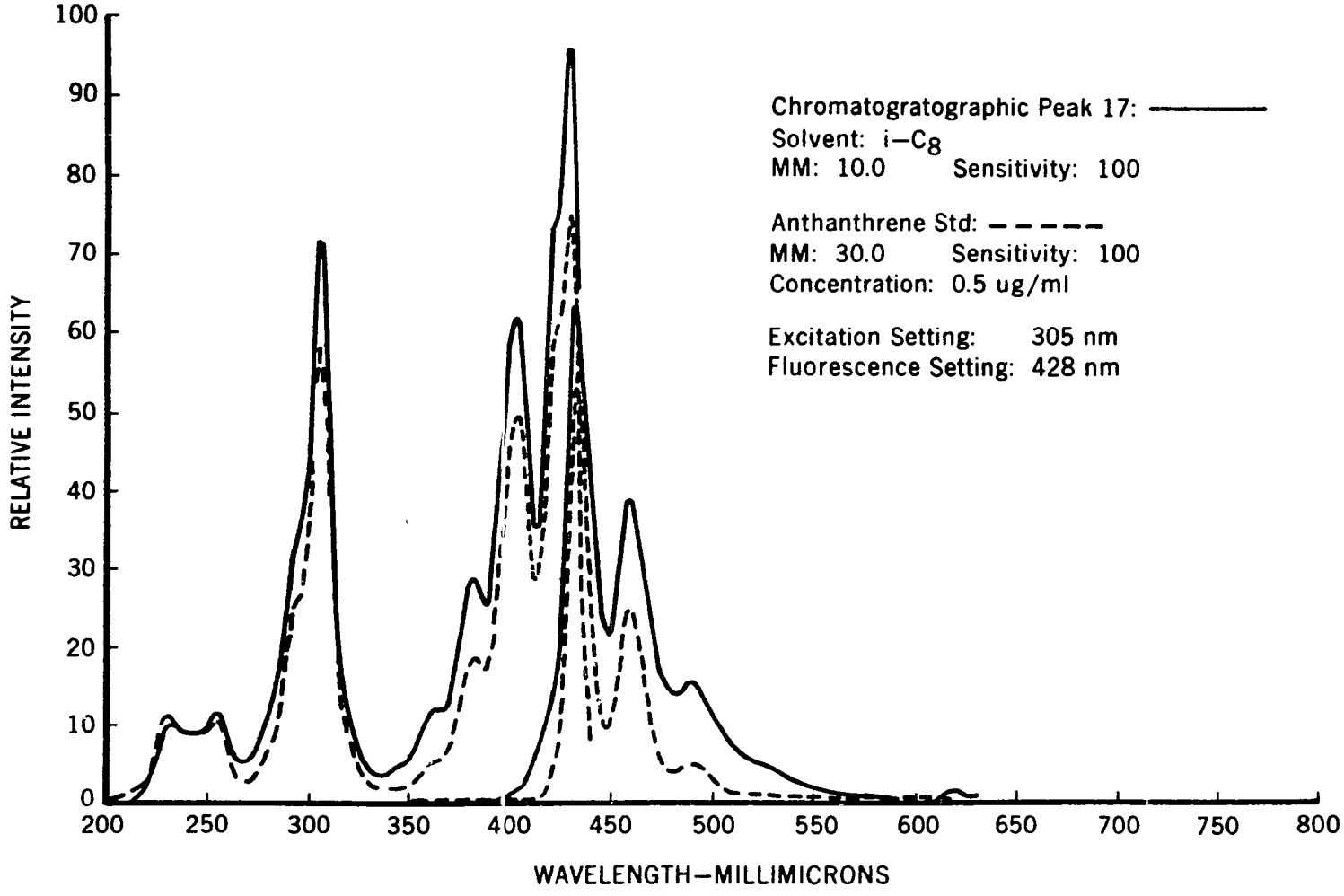
Excitation and Fluorescence Spectra of Chromatographic Peak 15 from SRF Carbon Black Extract and Perylene.



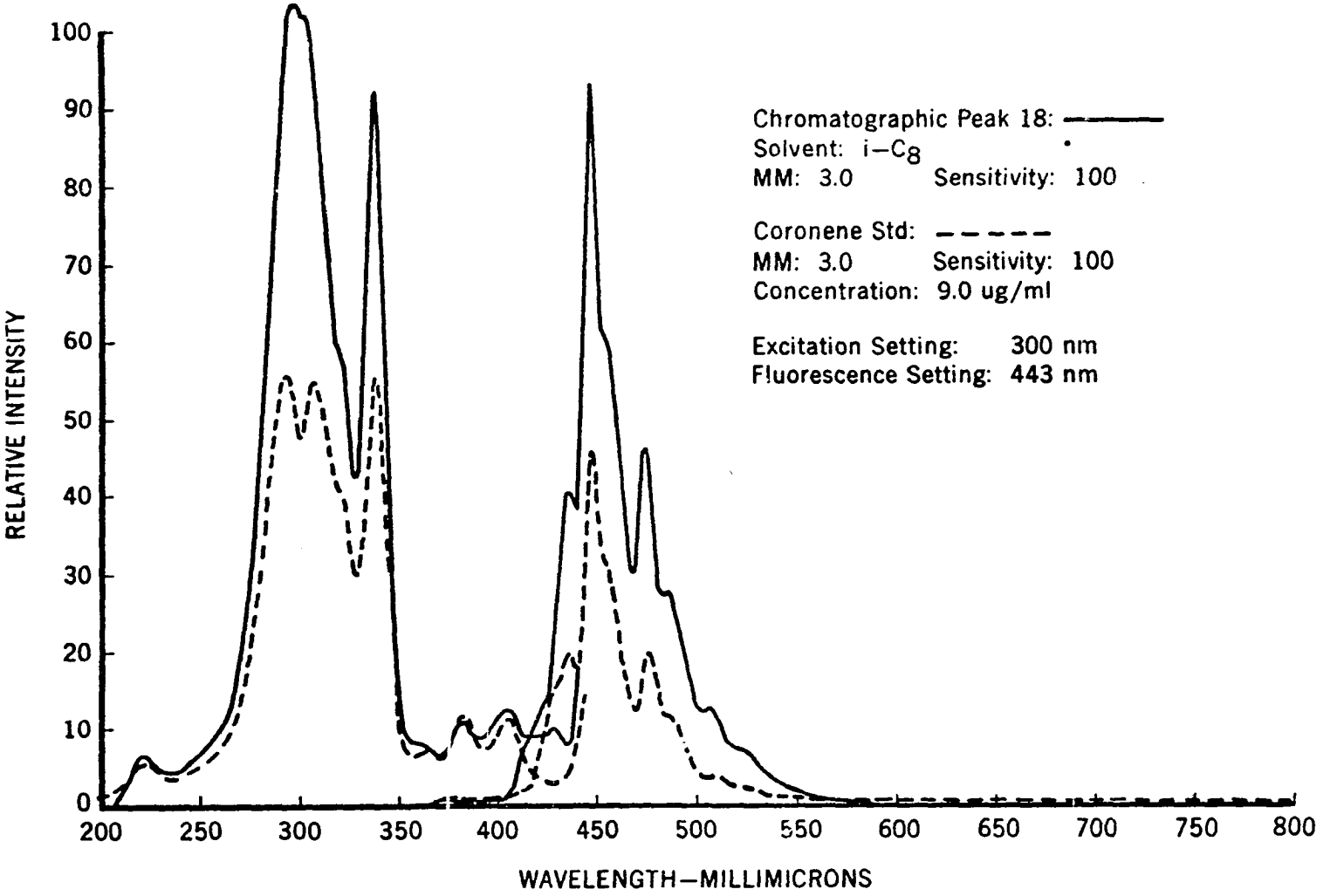
Excitation and Fluorescence Spectra of Chromatographic Peak 16 from SRF Carbon Black and 0—phenylene Pyrene.



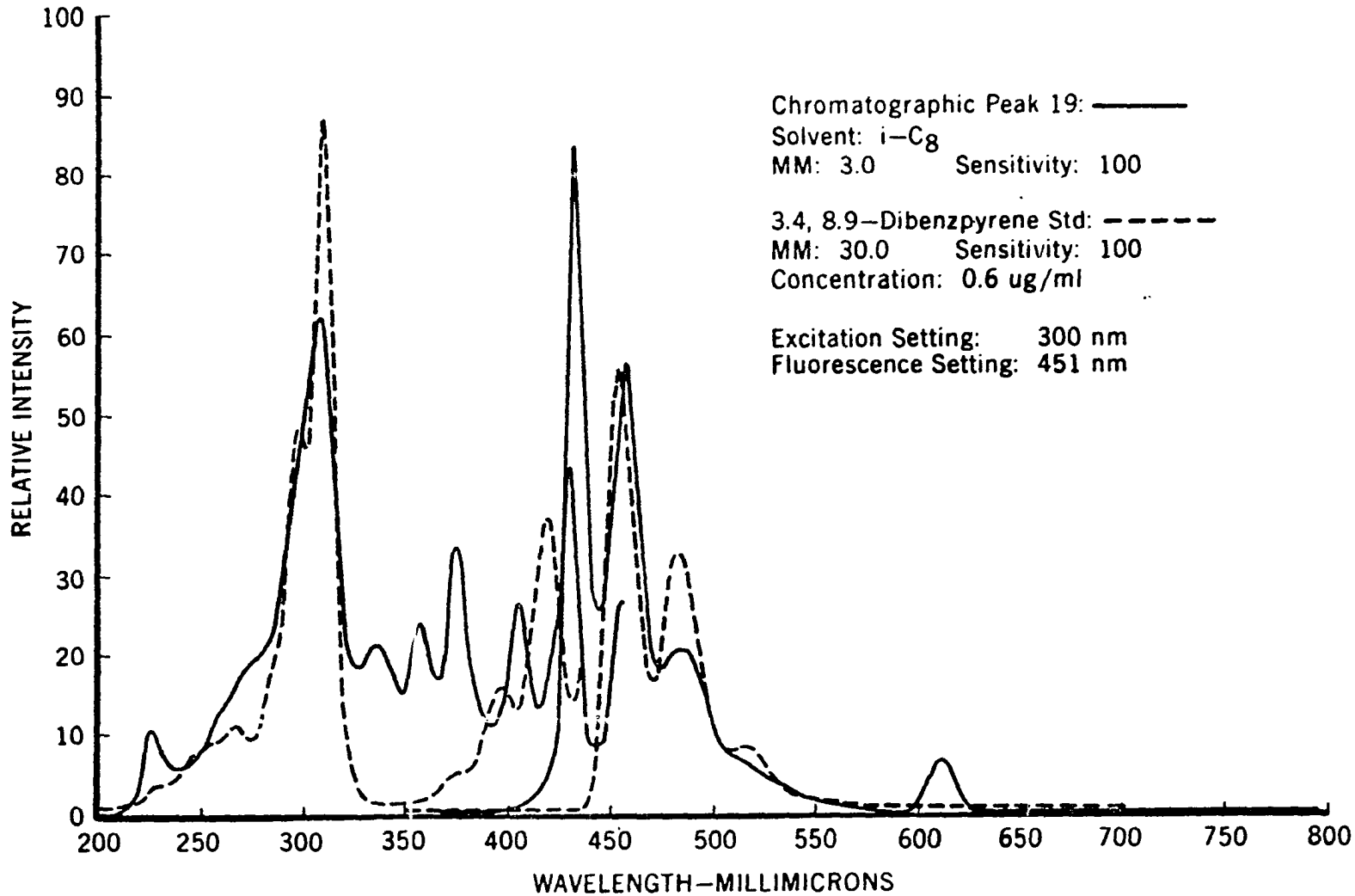
Excitation and Fluorescence Spectra of Chromatographic Peak 17 from SRF Carbon Black Extract and 1.12–benzoperylene.



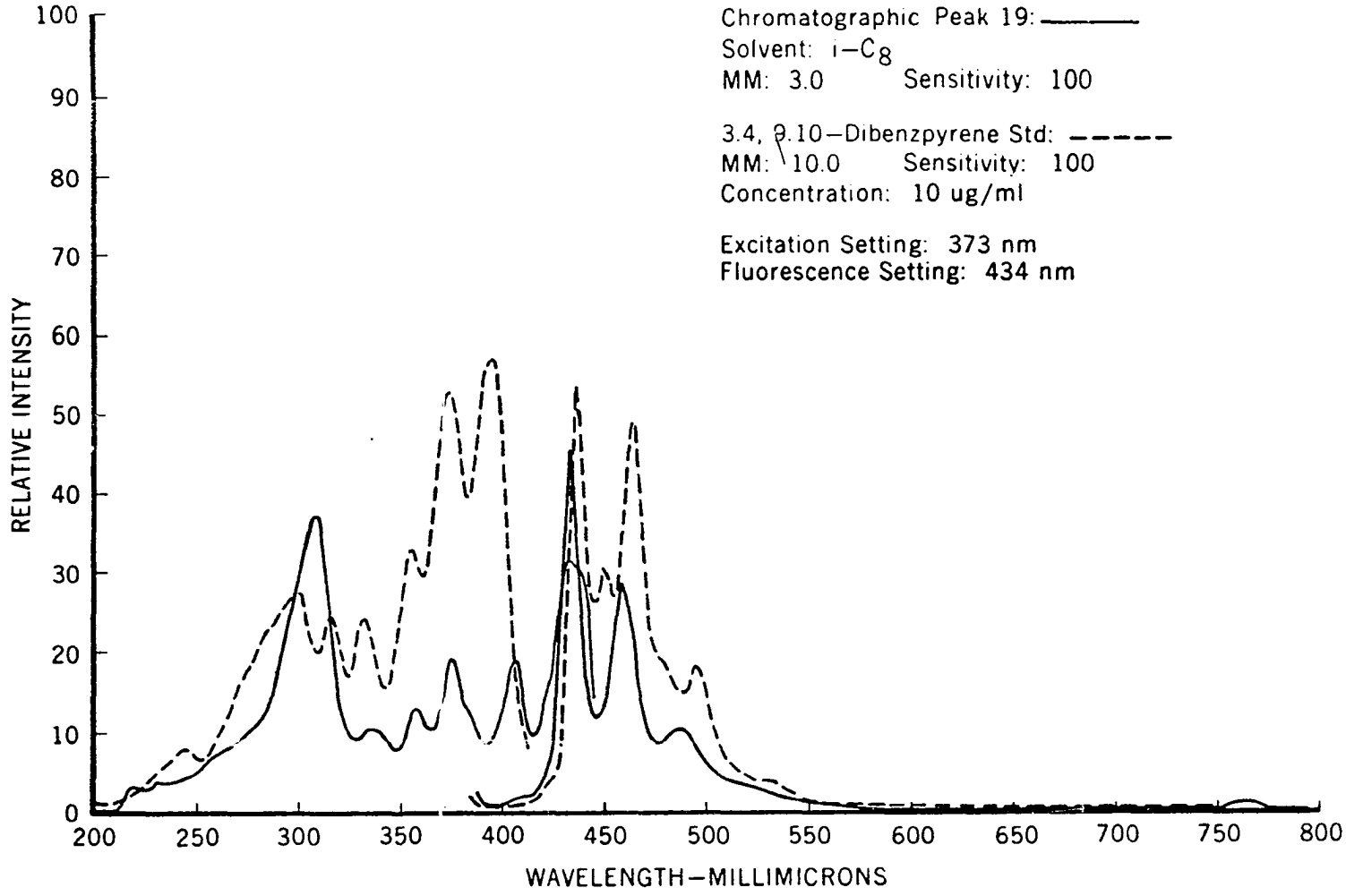
Excitation and Fluorescence Spectra of Chromatographic Peak 17 from SRF Carbon Black Extract and Anthanthrene



Excitation and Fluorescence Spectra of Chromatographic Peak 18 from SRF Carbon Black Extract and Coronene.



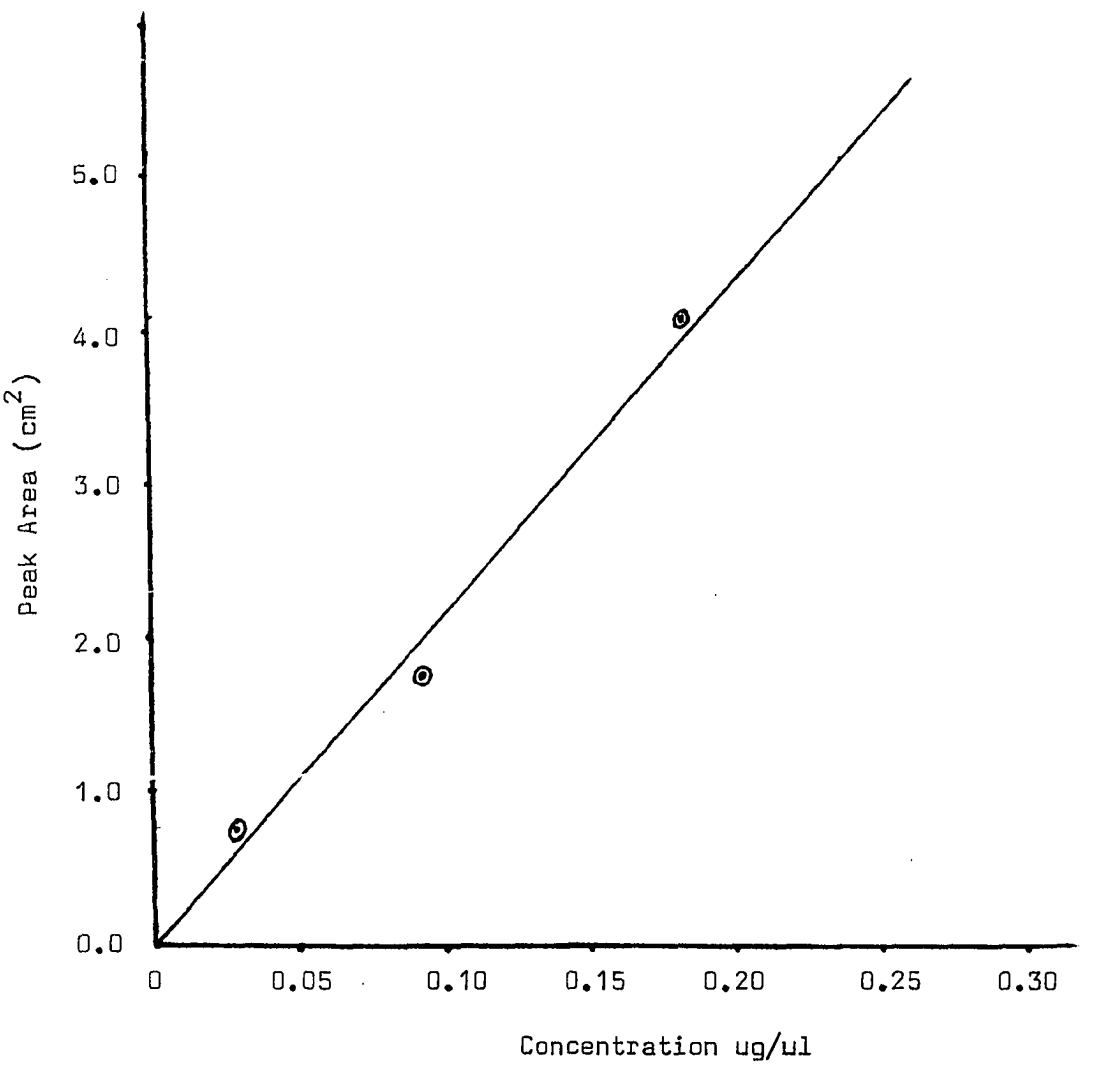
Excitation and Fluorescence Spectra of Chromatographic Peak 19 from SRF Carbon Black Extract and 3.4, 8.9–dibenzpyrene.



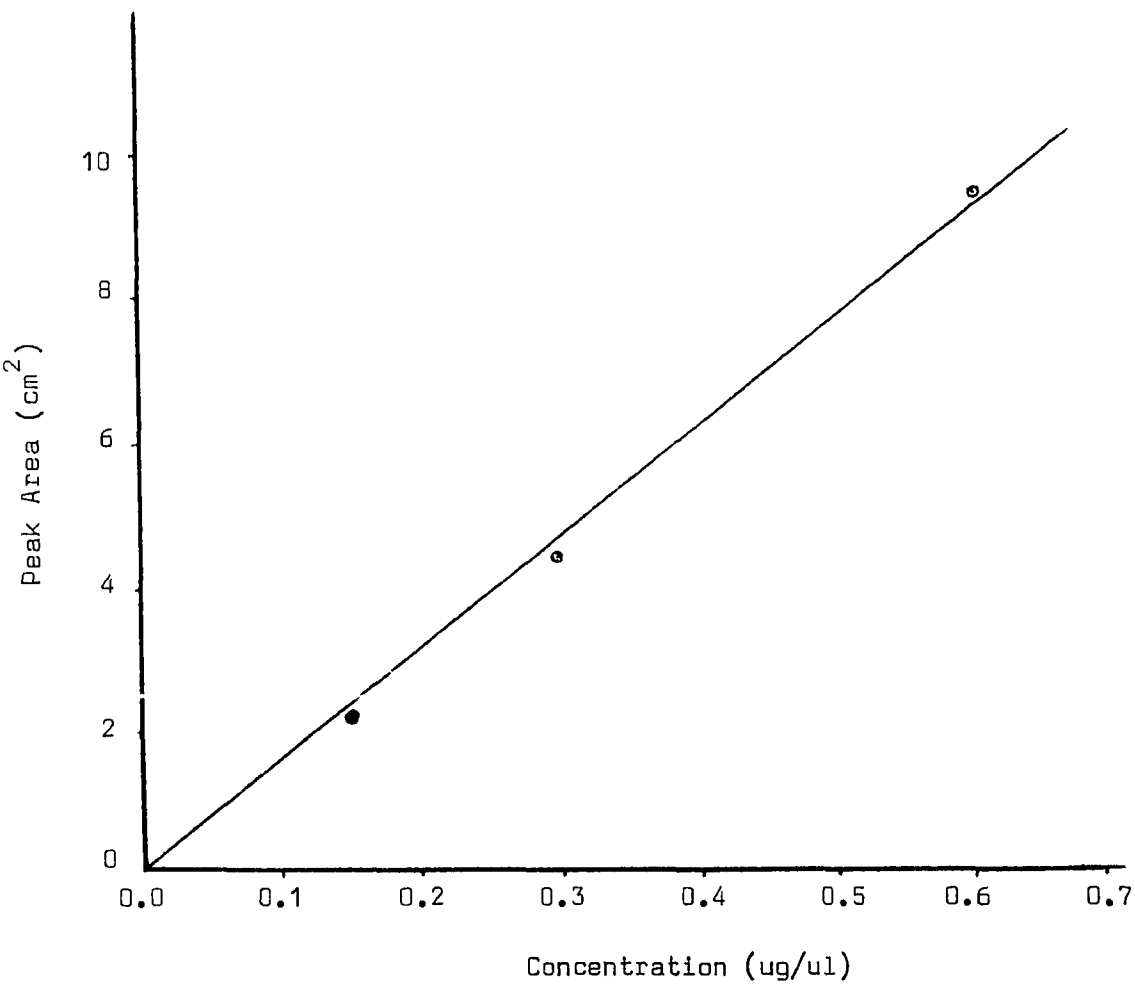
Excitation and Fluorescence Spectra of Chromatographic Peak 19 from SRF Carbon Black and 3.4, 9.10–dibenzpyrene.

APPENDIX D

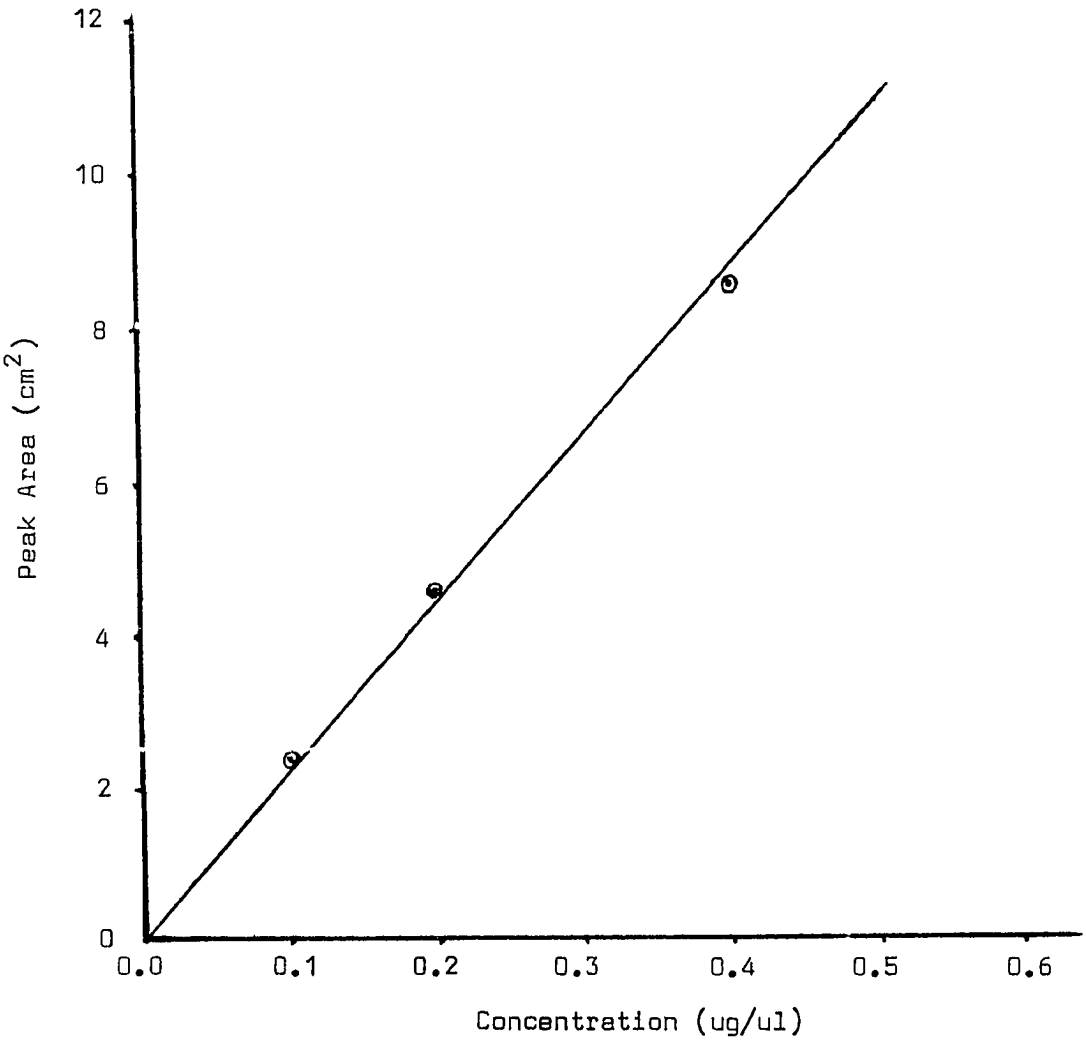
Standard Plots of Concentration against Peak Area
for Fluoranthene, DMBA, BAP, o-Phenylene
Pyrene and Coronene
Standards



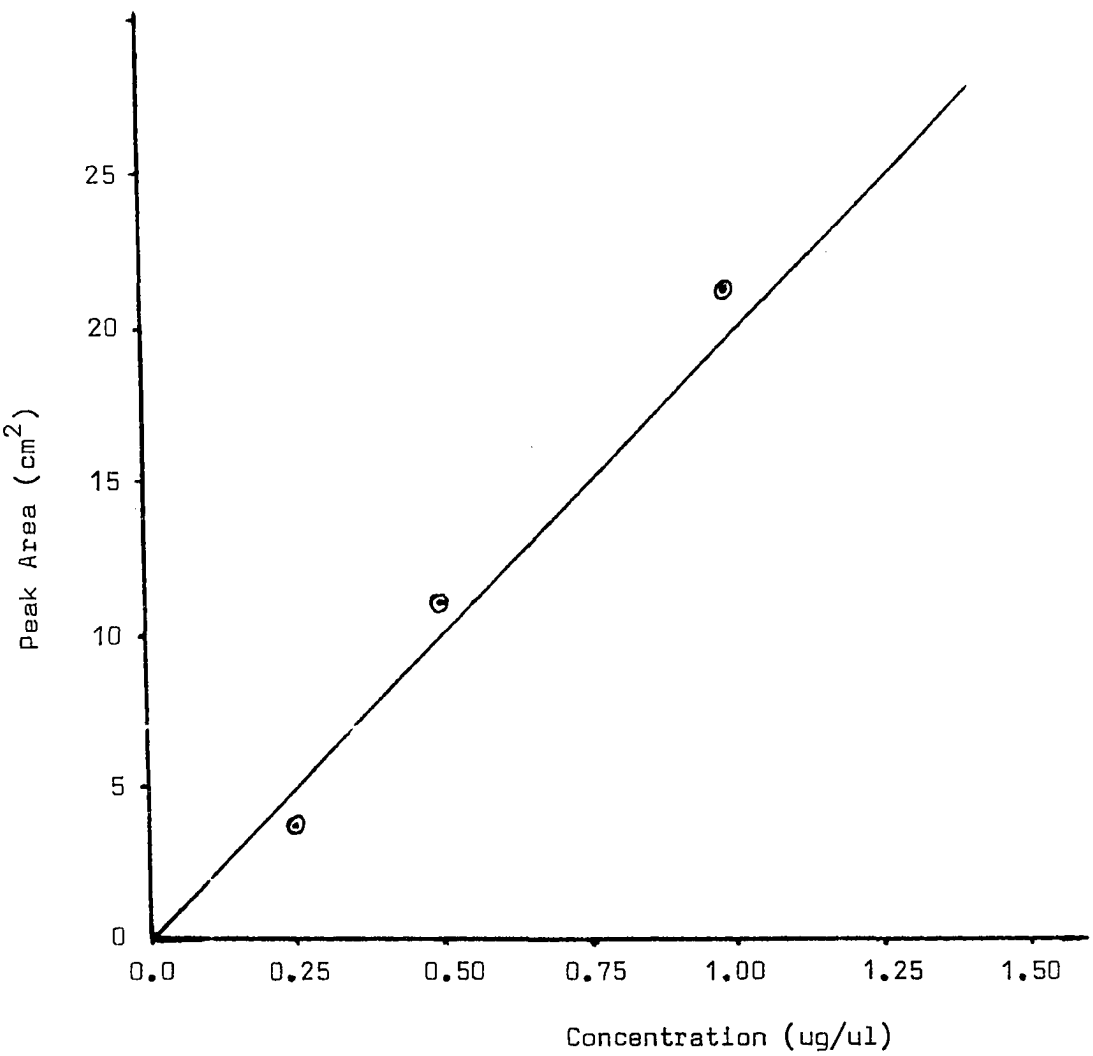
Plot of Concentration against Peak Area (at 200°C) for Fluoranthene.



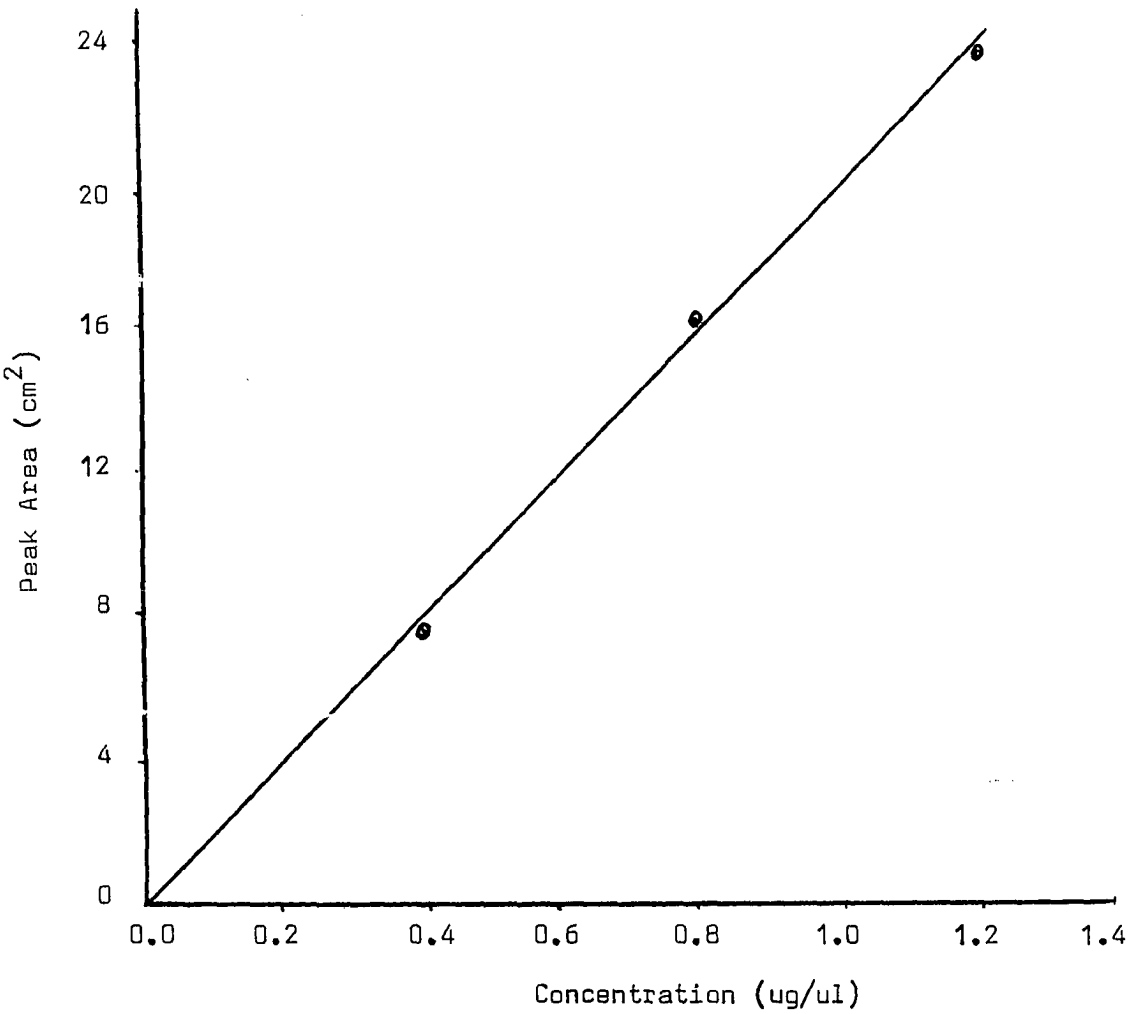
Plot of Concentration against Peak Area (at 200°C) for 9,10-Dimethyl-1.2-benzanthracene.



Plot of Concentration against Peak Area (at 250^{o}C) for 3.4-Benzopyrene.



Plot of Concentration against Peak Area (at 200^{o}C) for o-Phenylene pyrene.



Plot of Concentration against Peak Area (at 250^{o}C) for Coronene.