

DISCREET SEPARATION OF POLYCYCLIC HYDROCARBONS IN AIR BORNE PARTICULATES USING VERY LONG ALUMINA COLUMNS

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INTRODUCTION

Polycyclic hydrocarbons in recent years have become very important in air pollution studies; particularly so as several members of this family of compounds have demonstrable carcinogenic properties^{1,2}. The correlation between lung cancer mortality and the air borne concentrations of specific polycyclic hydrocarbons, 1:12-benzoperylene and 3:4-benzopyrene, has been statistically established in certain highly polluted areas of the industrial midland counties of Great Britain^{3,4}.

In work pioneered by KOTIN⁵, COOPER⁶, WEDGEWOOD AND COOPER⁷, LINDSEY *et al.*⁸, COMMINS⁹, KOTIN *et al.*¹⁰⁻¹² and others column chromatography on activated alumina or silica gel was used for primary division. However, the columns were generally of the order of three to six inches in length so that in many cases poor separation was achieved. This led to some excellent techniques of paper separation being devised, but these methods did not allow separation of the polycyclic compounds without at least some preliminary column separation.

The method outlined of long column separation then ultraviolet assay has proved very satisfactory for the discreet separation and identification of polycyclic compounds. Indeed, in many instances of quantitative estimation no other spectral media or chromatographic techniques are considered necessary.

APPARATUS AND MATERIALS

Activated alumina

Activated aluminium oxide, B.D.H. 100-200 mesh was heated in an air oven at 120°C for 72-96 h then brought to equilibrium over 50% sulphuric acid for at least 36 h. The moisture content of the alumina was then 13-13.5%.

Columns

A slurry of the alumina in cyclohexane was added to a 26.5 × 0.5 in. column to a depth of 23 in. with moderate vibration. Care was taken at all time to prevent the column becoming dry.

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Reagents

Where possible spectroscopic grade reagents were used. Because of the large volumes of cyclohexane required it was not economically possible to use this grade reagent so distillation followed by introduction of 5 g/l freshly activated alumina into the reagent bottles was adopted. A blank was then run on the purified reagent. This generally gave acceptable purity but in rare instances fractionation on a 1 in. diameter column packed to 16 in. with 3/16 in. raschig rings was necessary.

For the solid-liquid extraction, acetone, benzene or cyclohexane were used. For developing the column cyclohexane-ether was preferred.

Ultraviolet illumination

A Hanovia 3650 Å hand ultraviolet lamp was used to locate fluorescent zones on the column and to follow the passage of eluted material so that efficiency of separation and rate of elution could be systematised.

Ultraviolet spectrophotometer

A Beckman DK 2 split beam ratio recording spectrophotometer was used for ultraviolet spectral analysis of the eluates, with 3 ml quartz cells of 1 cm light path.

METHOD

Air particulate samples were obtained by passing 20-50 cubic ft./min through tared filter pads using a M.S.A. fixed flow, or Staplex variable flow, High Volume Air Sampler.

The filter pads of glass fibre or thick paper were weighed before and after the test against an equivalent standard pad and adjustments made for differential moisture content. This procedure was adopted because:

- (i) the thick paper pads absorb moisture at a fast rate if dried in an air oven to 105°C then brought to equilibrium in a desiccator, so that accurate weighing is impossible (with glass pads this would not be an objection);
- (ii) the loss of some polycyclic members such as fluorene is said to be appreciable if air dried at this temperature.

Where possible glass fibre pads dried before and after use in a desiccator are preferred but they have the drawbacks of low mechanical strength and low capacity.

The pads were extracted in a Soxhlet extraction apparatus with acetone solvent for about 5 h, the actual time being determined by a check on the fluorescence of the liquid condensed in the thimble portion. When an ultraviolet lamp showed this condensate to be free from fluorescent material, the extraction was stopped and the extracted liquid evaporated to dryness on a water bath to eliminate the acetone.

The solid residue was dissolved in cyclohexane, then transferred to the top of a 1/2 × 23 in. activated alumina column using a glass capillary tube fitted with rubber suction bulb and the minimum quantity of cyclohexane, certainly not exceeding 3 ml. Two circles cut from glass filter pad and inserted gently onto the top of the column prevented the alumina from being disturbed. Several loose strands of ether-washed "glass wool" fibres were placed on top of these discs.

The main eluting solvent, purified cyclohexane, was then run into the column

from a cylindrical separating funnel suspended above the column and 25 ml fractions collected with a LKB radirac fraction collector.

It was found that the columns were very sensitive to pressure (possibly due to the increased resistance encouraging pulsing of the bed) hence only gravity percolation was employed. After the first 8-10 samples gradually increasing amounts of ether were added to the cyclohexane up to a maximum of 30 %, the specific quantities being determined by the even elution of the various bands down the column. Passage of these bands was closely followed with a 3650 Å ultraviolet lamp, so that care could be exercised in keeping the zones horizontal and sharply defined, hence systematising the ether additions.

Total samples numbered seventy to one hundred and twenty. These samples were transferred to brown phials then evaporated on a low temperature water bath to eliminate the ether and concentrate the samples.

Each fraction was separately run on a Beckman DK2 Ratio Recording Ultraviolet Spectrophotometer.

The order of elution of the polycyclic compounds is constant and this fact vastly simplifies systematic enquiry of unknown fractions. In Table I the relative elution rate of some of these compounds is given.

TABLE I
ORDER OF ELUTION OF POLYCYCLIC COMPOUNDS
ON ACTIVATED ALUMINA WITH CYCLOHEXANE-ETHER SOLVENT

Aliphatics
Olefins
Benzene, toluene and derivatives
Naphthalene and naphthalene derivatives
Acenaphthene
Fluorene
Phenanthrene
Anthracene
Pyrene
Fluoranthene
1:2-Benzofluorene and 2:3-benzofluorene
9:10-Benzophenanthrene
1:2-Benzanthracene
Chrysene
3:4-Benzopyrene
1:2-Benzopyrene
Perylene
2:3-Benzofluoranthene
1:12-Benzoperylene
Anthanthrene
Coronene

A library of standard substances has been compiled allowing standard solutions of known concentrations in cyclohexane to be made. The graphs of these standards besides characterising and identifying polycyclic compounds enable quantitative measurements to be made using the Beer-Bouguer law of linear concentration/absorbance dependence.

The importance of graphing the actual standard substance under identical conditions to the run of unknowns cannot be too highly stressed. For the quantitative

determinations use was made of a local baseline technique, an extension of the work of COOPER⁶, particularly where background absorbance interfered. When this effect did occur it was more evident at wave lengths from 280 m μ to the air absorbance at 180 m μ .

Generally the range of wave length values was 440–210 m μ the technique of scale magnification being often adopted particularly in the range 440–360 which precedes a cam change on the Beckman DK2 spectrophotometer.

Paper chromatography may sometimes be used to help resolve badly mixed fractions. The solvent systems acetic acid–ether–water (12:5:20) and *n*-propanol–ether–water (20:9:4) on acetylated paper, Carl Schleicher and Schüll 2043b mgf, proved the most satisfactory. SPOTSWOOD'S system¹³ ethanol–toluene–water (17:4:1) and that of BERGMANN AND GRUENWALD¹⁴ methanol–toluene–water (10:1:1) were also used.

R_F values for the acetic acid–ether–water system are given in Table II.

TABLE II
 R_F VALUES ON ACETYLATED PAPER S & S 2043b WITH SOLVENT
ACETIC ACID–ETHER–WATER (12:5:20)

Compound	R_F value	Colour with 253.7 m μ ultraviolet light
3:4-Benzopyrene	0.43	purple
1:2-Benzopyrene	0.33	purple
Fluoranthene	0.88	intense white-blue
1:9-Benzanthrone	0.81	intense olive green
1:2-Benzanthracene	0.56	violet
Pyrene	0.74	deep royal blue
Phenanthrene	0.77	faint blue-brown
Chrysene	0.71	ochre
3:4-Benzofluorene	0.66	royal blue
1:2-Benzofluorene	0.57	royal blue
Perylene	0.34	light blue
1:12-Benzoperylene	0.26	purple
1:2;3:4-Dibenzopyrene	0.15	lemon yellow
3:4;9:10-Dibenzopyrene	(0.05)	white-blue
	(0.18)	brown
1:2;3:4-Dibenzanthracene	0.22	purple
3-Methylcholanthrene	0.28	violet
Phenanthraquinone	0.88	light brown (visible light)
Pentacene	0.26	fawn

Some mixing occurs at times between polycyclic compounds adsorbed to about the same degree. This is most prevalent with the compounds benzophenanthrene–benzanthracene–chrysene but only in the rare case where the concentration of benzanthracene is small compared to the other two tetracyclic isomers do all three appear on the one graph. Even when this effect occurs it is only over a small range and can be resolved quantitatively by three-dimensional analysis. Normally the benzophenanthrene–benzanthracene and benzanthracene–chrysene couplets allow ready quantitation by two-dimensional techniques.

Typical analysis of an air sample will emphasise the above arguments.

The sample, on the roof of Sydney Town Hall was collected by passing 422,500 cubic ft. of air at 50 cubic ft./min to give a total solid deposit of 2.011 g. After extraction and column chromatography 87 fractions were obtained.

SYSTEMATIC ANALYSIS

Benzene, toluene and derivatives. Shown in fractions 5,6. Characteristic wave lengths benzene 268 (slight), 261, 255, 248, 244 $m\mu$; toluene 268, 265 S, 262, 255, 248, 244 $m\mu$.

Acenaphthene. Fractions 16, 17 (mainly) 18. Wave length maxima 229 (very strong), 280, 289, 301, 307, 321 $m\mu$.

Fluorene. Trace in fraction 21. Wave length maxima 255, 262, 272 $m\mu$.

Phenanthrene. Trace in fraction 22 mixed with anthracene. Wave lengths 244, 251, 258 S, 264 S $m\mu$. Presence not confirmed by chromatography on acetylated paper with acetic acid-ether-water (12:5:20).

Anthracene. Fractions 22 and 23. Wave length maxima 221, 246 S, 252 $m\mu$.

Pyrene. Fractions 24, 25, 26, 27 (mixed). Very characteristic spectra at 231, 238 S, 241, 252, 262, 273, 306, 319, 336 $m\mu$. Fig. 1 shows sample 25.

Fluoranthene. Fractions 27, 28, 29. Very characteristic spectra at 236, 243 S, 249, 263, 271 S, 277, 288 $m\mu$. Fraction 28 is shown in Fig. 2.

1:2-Benzofluorene and 2:3-benzofluorene. Only a trace is evident in fractions 31, 32. The fractions concentrated to 1 ml were chromatographed on acetylated paper with acetic acid-ether-water (12:5:20) and a faint trace of these compounds appeared to be present.

9:10-Benzophenanthrene. Fractions 40, 41, 42 (mixed with benzanthracene). Wave length maxima 250, 260, 275, 286 $m\mu$. Quantitative estimation of fraction 42 requires two-dimensional analysis. Fig. 3 shows sample 40.

1:2-Benzanthracene. Mixed with benzophenanthrene in sample 42, with chrysene in samples 43, 44. Diagnostic wave lengths 226, 256, 277, 298, 316 $m\mu$. See Fig. 4 showing sample 42, Fig. 5, sample 43.

Chrysene. Fractions 43, 44, 45, 46, 47, 48. Pertinent wave lengths 241, 250 S, 259, 269, 282, 295, 306, 321. Fig. 6 shows sample 46.

3:4-Benzopyrene and 1:2-benzopyrene. Together in fractions 50, 51, 52. 3:4-Benzopyrene is readily identified by magnifying scale in region 360-450 to give diagnostic wave lengths at 385 $m\mu$, 403 $m\mu$. The 403 peak is used for quantitative estimation of 3:4-benzopyrene. See Figs. 7, 8.

2:3-Benzofluoranthene. Evident in fractions 59, 60. Wave length maxima 257, 263 S, 277, 282 S, 290, 294 S, 303 $m\mu$. Fraction 59 is shown in Fig. 9.

1:12-Benzoperylene. Fractions 61, 62, 63, 64, 65. Characteristic wave lengths 250, 259.5, 275, 286 $m\mu$. Fig. 10 compares pure 1:12-benzoperylene with sample 63.

Anthanthrene. A quantity evident in fractions 69, 70. Fluorescent spectra using a mercury lamp and a Corning filter 5840 to radiate the fluorescent cell at 365 $m\mu$ gave wave length maxima at 426, 434, 456 and 488 $m\mu$.

Coronene. The sample of coronene was spread over samples 70 (mixed) 71, 72, 73, 74, 75, 76. A comparison between sample 73 and pure coronene is given in Fig. 11.

Concentration of the polycyclic hydrocarbons on a weight and a volume basis are given in Table III.

TABLE III
 CONTENT OF POLYCYCLIC HYDROCARBONS,
 ATMOSPHERIC SAMPLE, SYDNEY TOWN HALL

<i>Compound</i>	<i>Hydrocarbons p.p.m. of solid</i>	<i>Hydrocarbons μg/100 m³</i>
Acenaphthene	3.9	0.07
Anthracene	2.8	0.05
Pyrene	15.2	0.26
Fluoranthene	17.9	0.30
9:10-Benzophenanthrene	14.2	0.24
1:2-Benzanthracene	3.5	0.06
Chrysene	20.1	0.34
1:2-Benzopyrene	18.8	0.32
3:4-Benzopyrene	20.7	0.35
2:3-Benzofluoranthene	3.0	0.05
1:12-Benzoperylene	21.8	0.37
Anthanthrene	8.0	0.13
Coronene	15.9	0.27

DISCUSSION

Column adsorption chromatography is undoubtedly the best means of initial separation of the polycyclic mixture. Figs. 1-11 are actual photographs of a run of unknown fractions graphed on the paper supplied with the Beckman spectrophotometer. Spectra of the pure compounds are superimposed on these graphs and are indicated by a broken or dotted line. The clarity of separation and purity of the compounds relative to the standard substances is evident. Although with proper care such good separation is obtained, the method is lengthy and often some compromise must be reached between clear separation and elution time. For more routine investigations where five or six of the more common polycyclic compounds are required the column length could be cut to say 10 in. allowing comparable care of elution.

As air samples are much more complex than the pure polycyclic compounds which give such good results on paper, at least some arbitrary fractionation on alumina is required. If only qualitative data is wanted, paper chromatography from this point may well be used. SPOTSWOOD'S method¹³ of activated cellulose columns with solvent systems analogous to those used in paper chromatography has advantages over the normal paper methods in the far larger surfaces available for adsorption. For quantitative analysis of such samples, however, the good initial separation obtained by long alumina columns is very necessary.

With very impure samples of a tarry nature the method of CAHNMANN AND KURATSUNE¹⁵, who partitioned polycyclic compounds between cyclohexane and aqueous methanol (1:4) was tried. The partition coefficients cyclohexane phase/water-methanol (1:4) phase for the polycyclic hydrocarbons found in the extracts varied from 14 to 150. A further partition between cyclohexane and nitromethane with coefficients nitromethane phase/cyclohexane phase from 1.5 to 4.4 was suggested by HOFFMANN AND WYNDER¹⁶. However, with both these methods there was often a carryover into the cyclohexane phase of unknown substances which poisoned the alumina, preventing good separation. Preliminary purification of tarry samples on 12 in. alumina columns with repeated ether washing appeared to be the best solution.

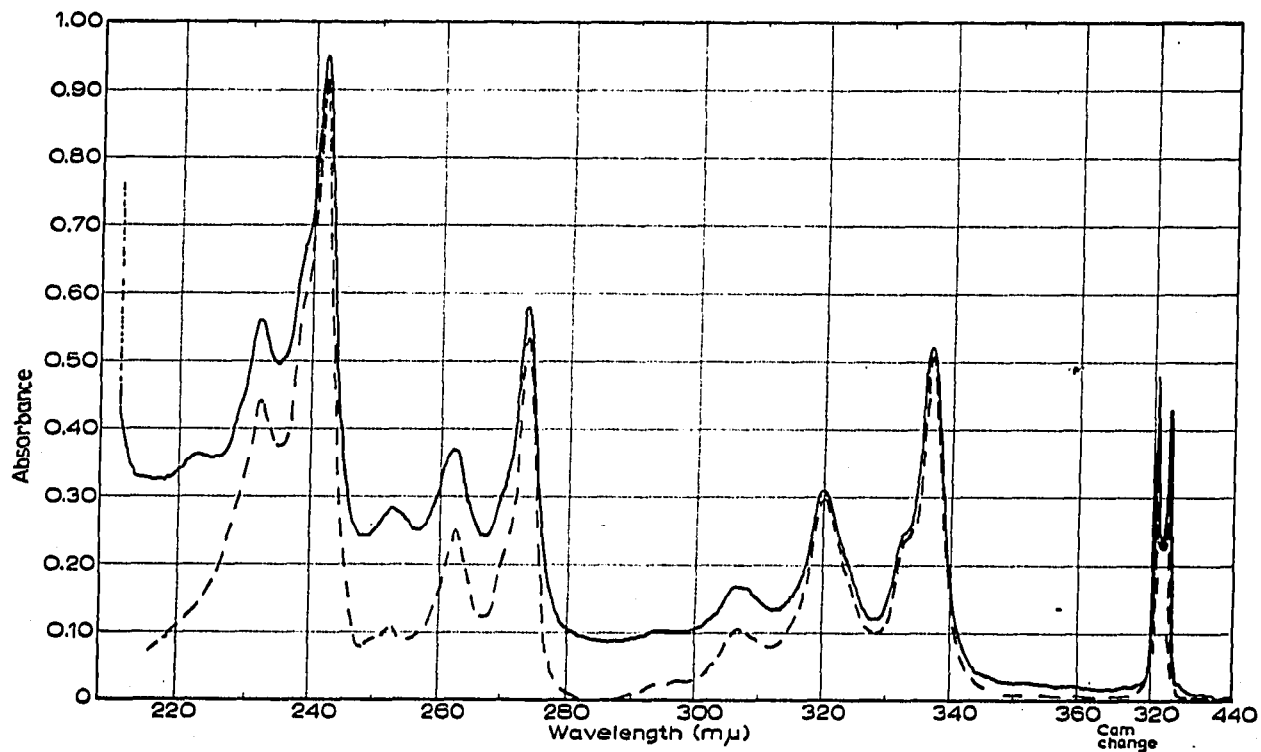


Fig. 1. Sample 25 (—); pyrene (---).

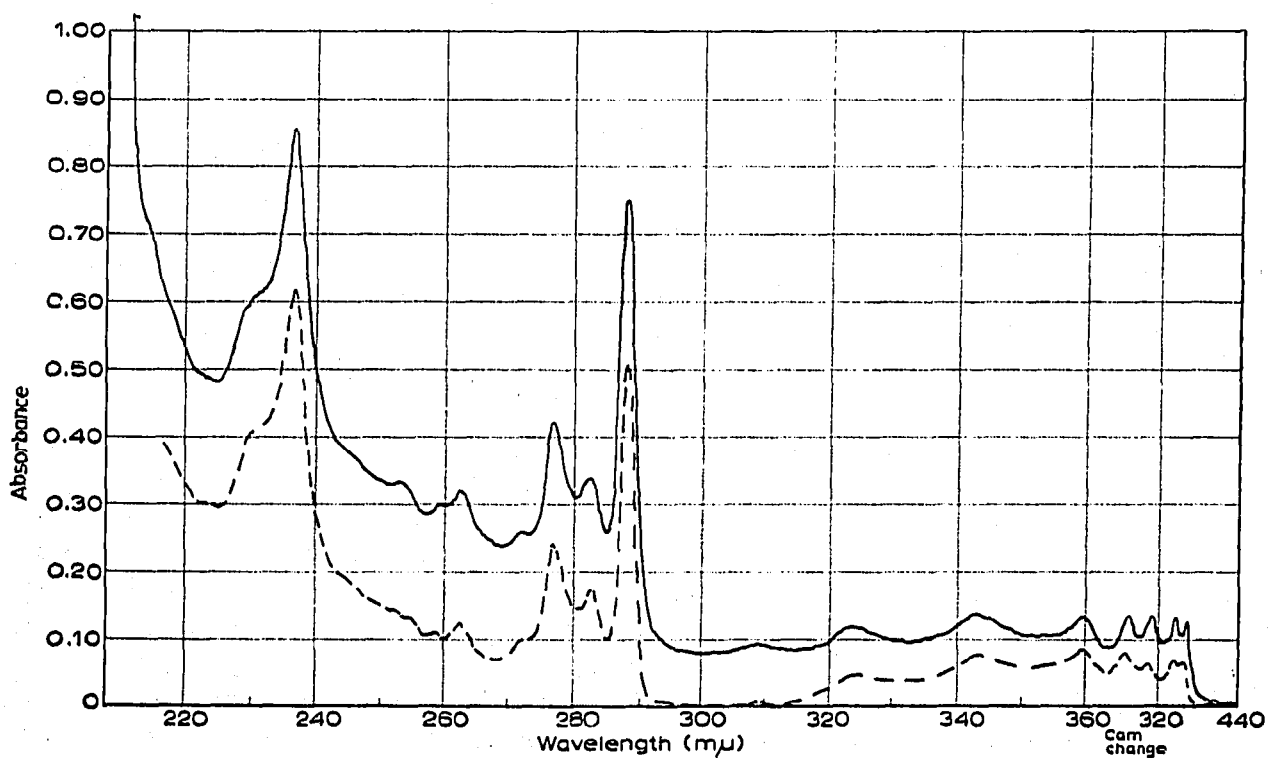


Fig. 2. Sample 28 (—); fluoranthene (---).

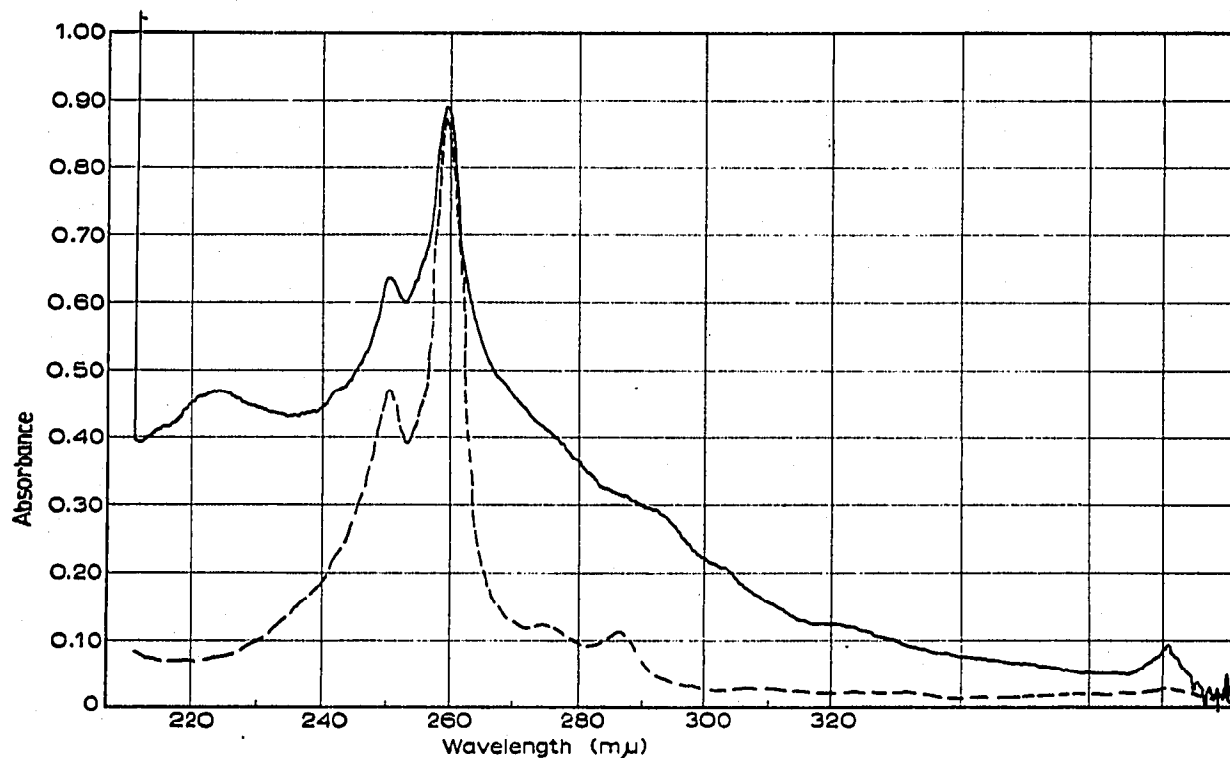


Fig. 3. Sample 40 (—); 9:10-benzophenanthrene (---).

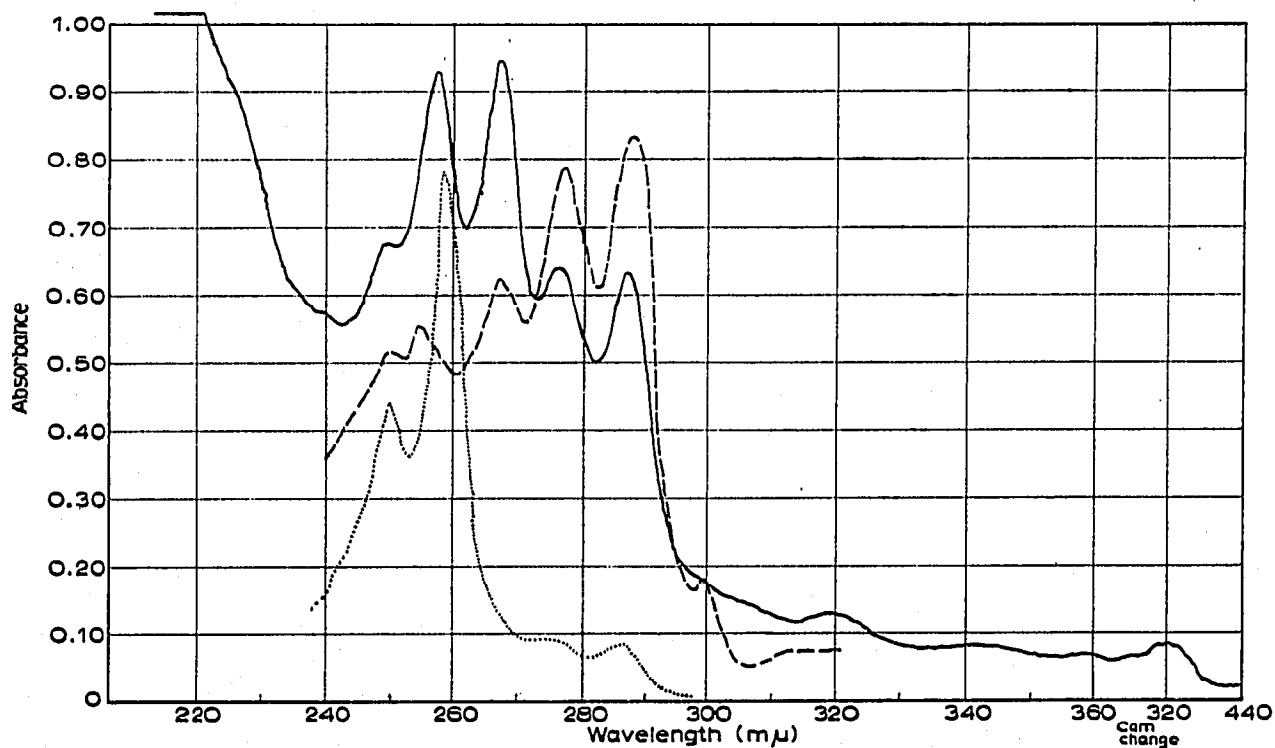


Fig. 4. Sample 42 (—); 9:10-benzophenanthrene (···) and 1:2-benzanthracene (---).

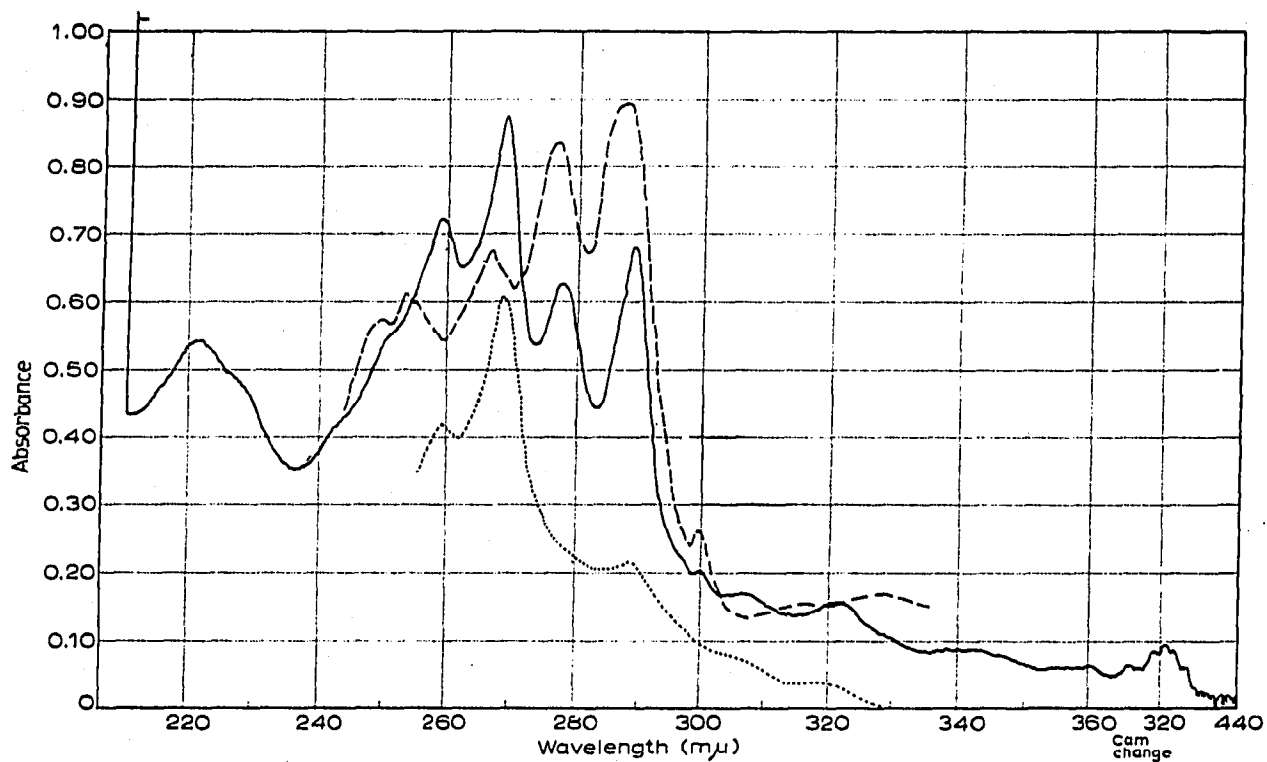


Fig. 5. Sample 43 (—); 1:2-benzanthracene (---) and chrysene (···).

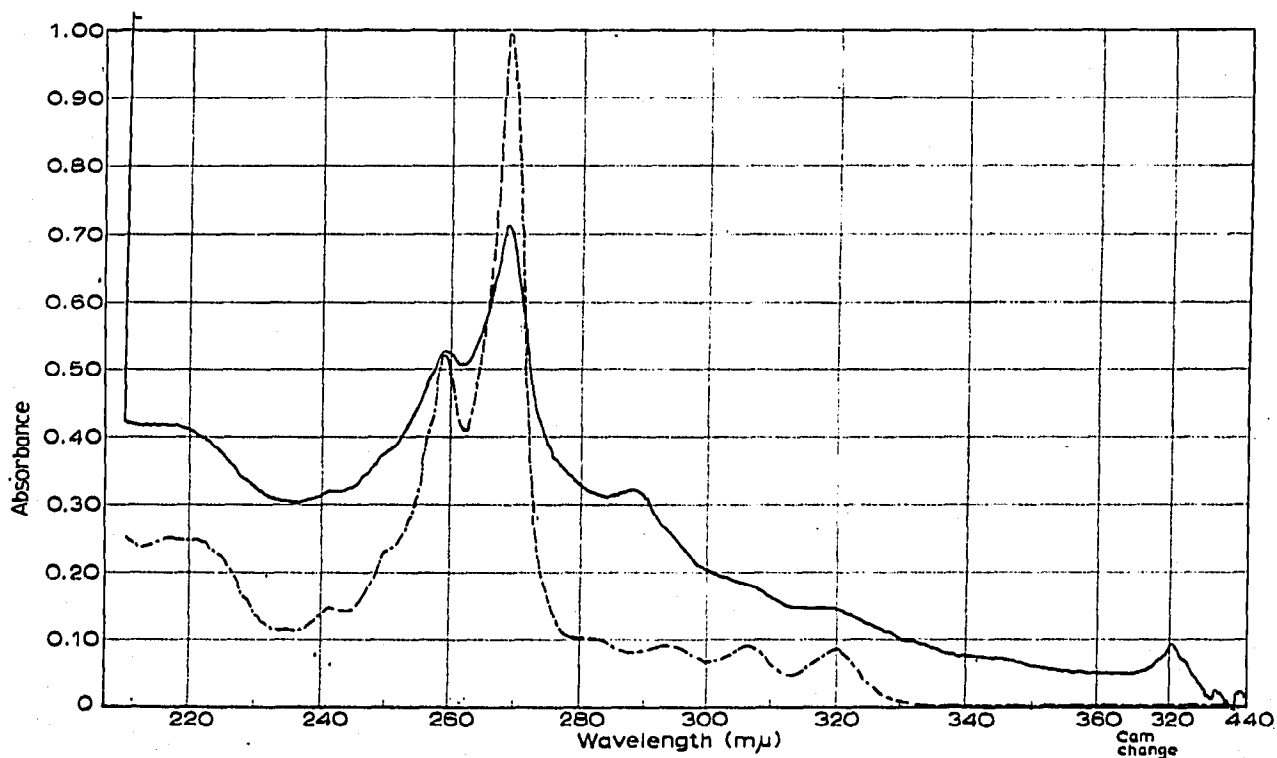


Fig. 6. Sample 46 (—); chrysene (---).

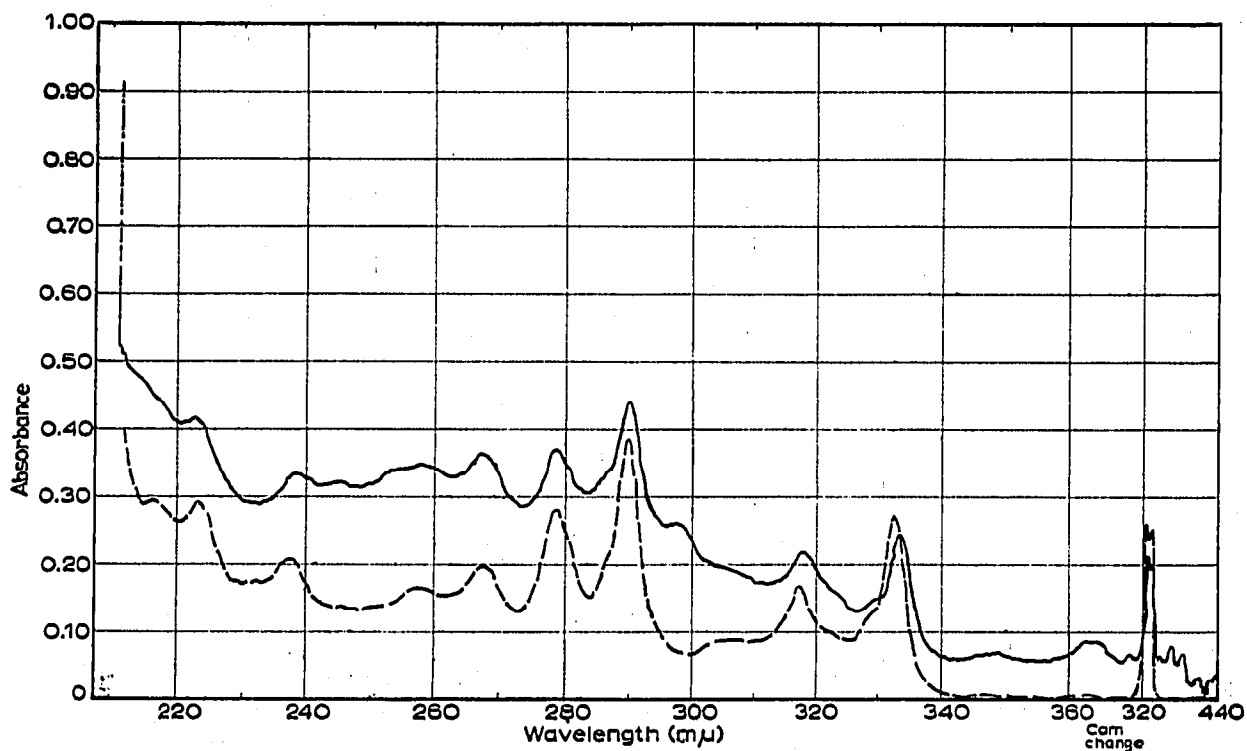


Fig. 7. Sample 51 (—); pure 1:2-benzopyrene (---).

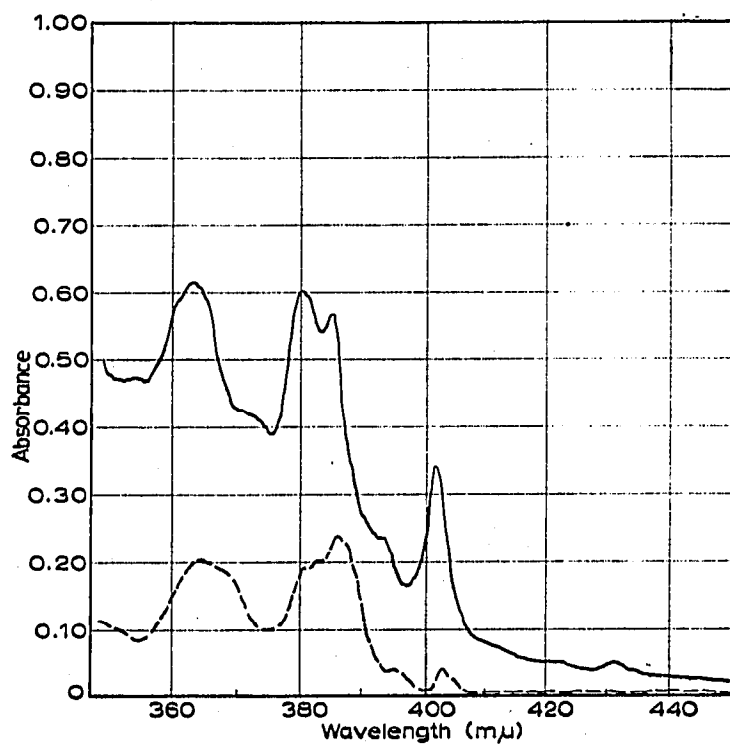


Fig. 8. Sample 52 (—); pure 3:4-benzopyrene (---).

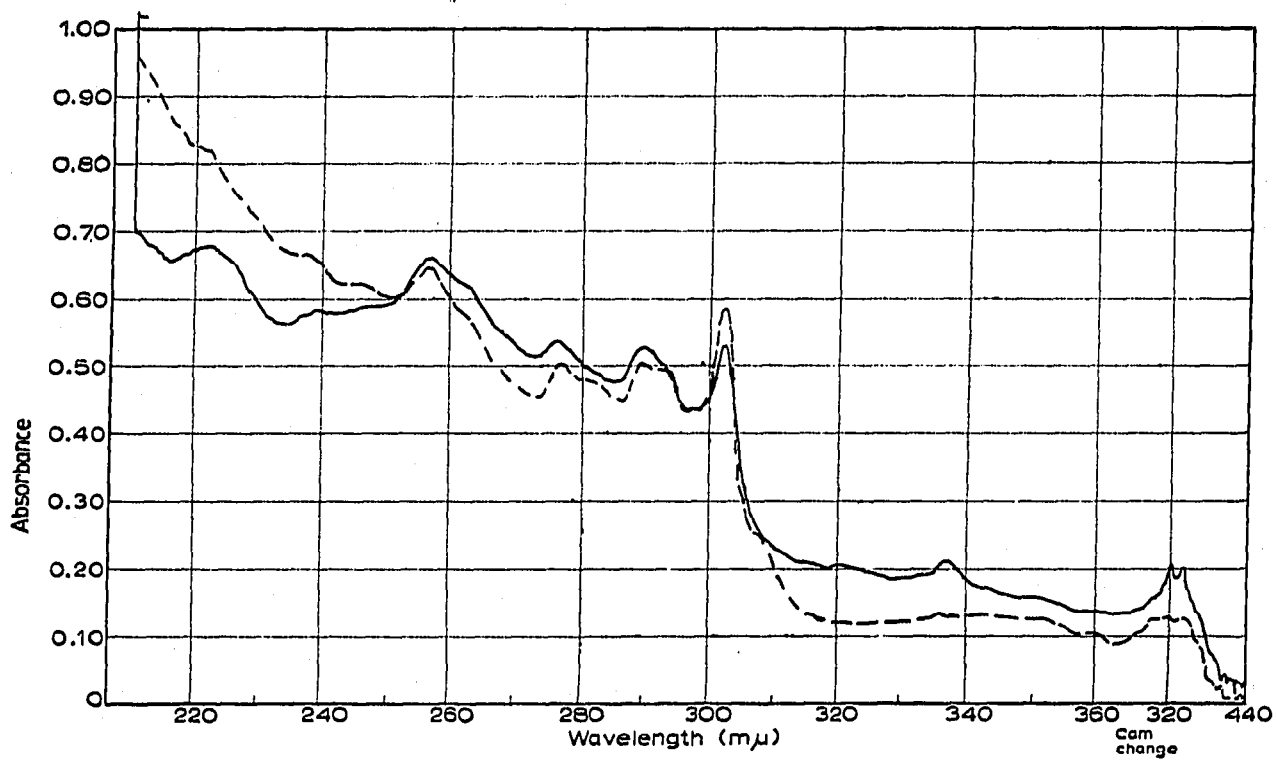


Fig. 9. Sample 59 (—); 2:3-benzofluoranthene (--).

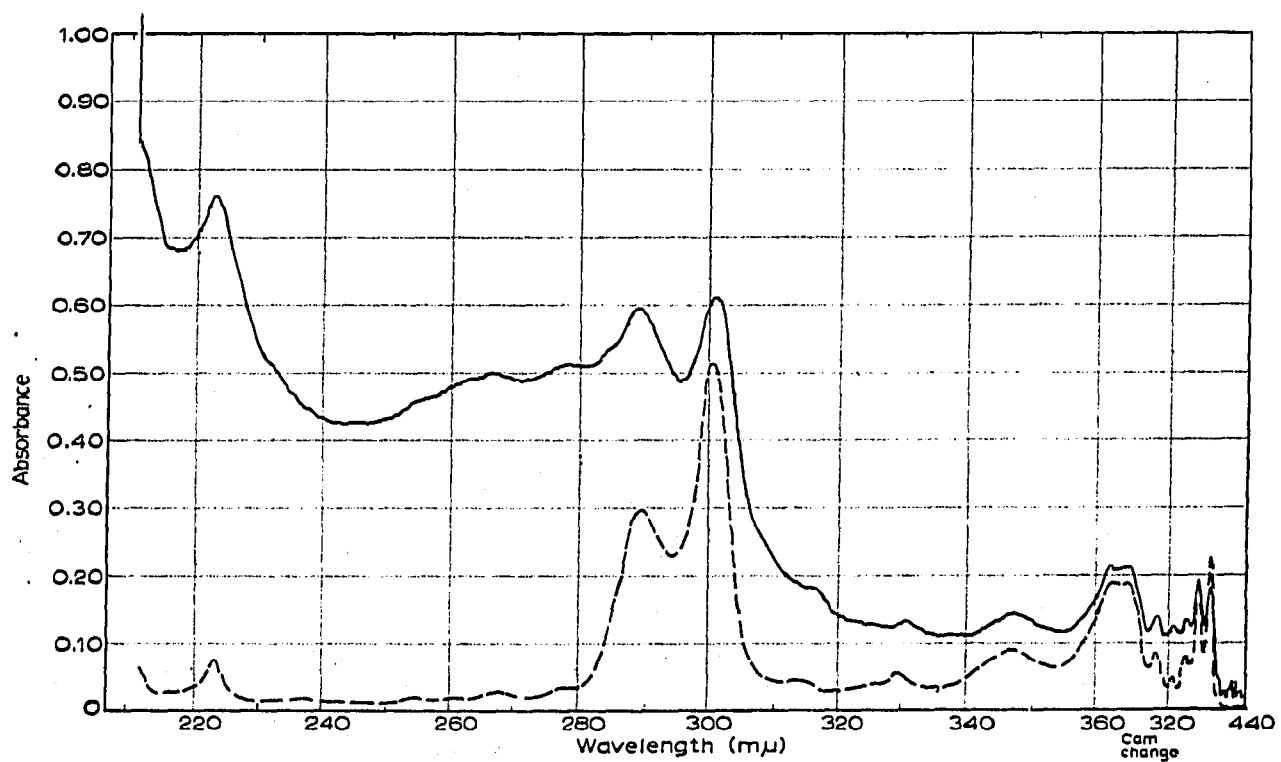


Fig. 10. Sample 63 (—); 1:12-benzoperylene (--).

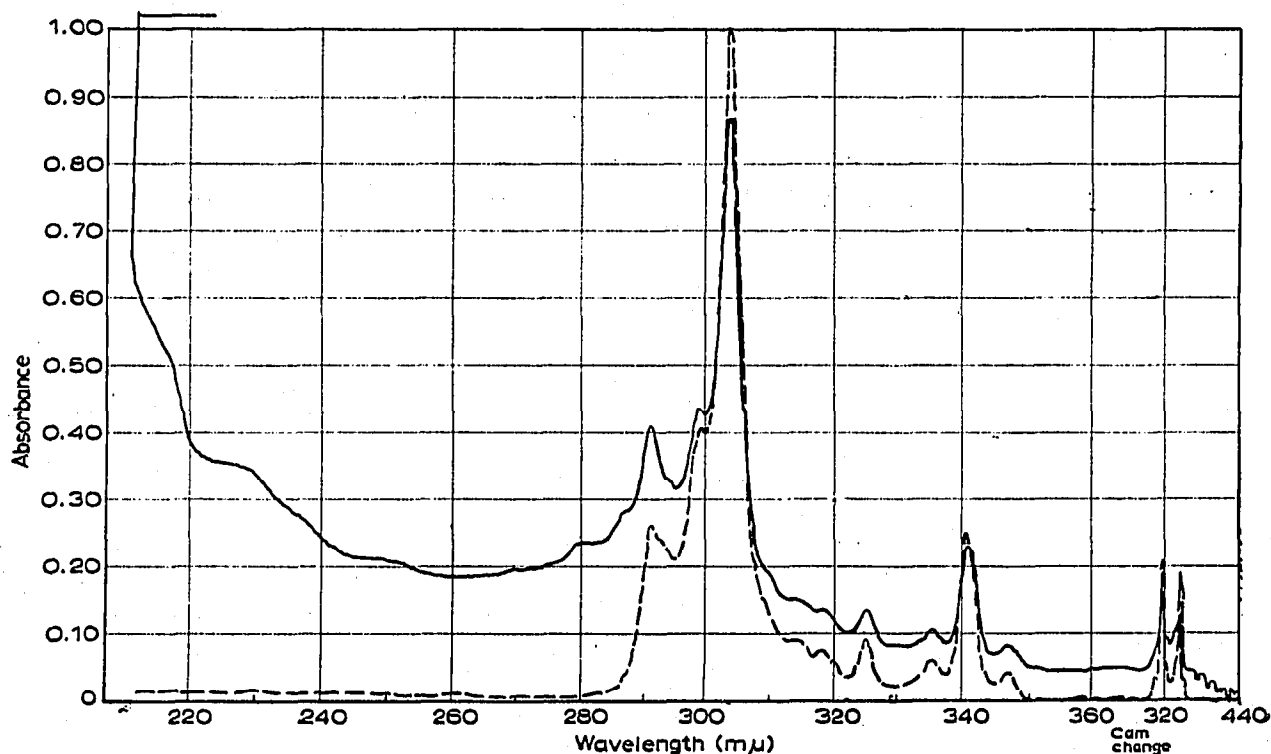


Fig. 11. Sample 73 (—); coronene (---).

SUMMARY

Use has been made of an unusually long alumina column to obtain good separation of the complex polycyclic fraction of air particulates.

In many instances of quantitative estimation no other spectral media or chromatographic technique other than ultraviolet spectroscopy need be contemplated.

An example has been given of the chromatographic separation and identification of a typical air pollution sample.

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