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STUDIES OF THE APPLICATION OF SELECTIVE CHROMATOGRAPHIC AND SPECTROFLUORIMETRIC TECHNIQUES IN THE SEPARATION, CHARACTERIZATION, AND ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS

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SUMMARY

A natural complex mixture of polycyclic aromatic hydrocarbons (PAHs) has been separated by three selective stages, namely preparative-scale chromatography on Sephadex LH-20, high-performance liquid chromatography (HPLC) of the fractions on Partisil 5, and finally thin-layer chromatography (TLC) of HPLC peaks on a Bentone 38 layer using a novel microslide technique followed by automatic fluorimetric scanning of the plate. The different retention effects are discussed in relation to PAH structure and these have been utilized in the characterization of many of the components. Spectrofluorimetry was used for detection and identification and the advantages of this technique with respect to selectivity and also as a further aid to component characterization are discussed and illustrated with reference to some of the important PAH components.

A novel TLC procedure is described in which compounds such as benzo[*a*]-pyrene can be determined rapidly in environmental samples by direct transfer of the separated zones into toluene solution and measurement of the fluorescence spectrum.

INTRODUCTION

The need to develop reliable methods for the analysis of polycyclic aromatic hydrocarbons (PAHs) arises from their well-known toxicological properties and their ubiquitous occurrence in the environment. Considerable research effort has been devoted to this topic in recent years, mostly in relation to the application of chromatographic techniques coupled with methods for the selective detection and identification of components. The most successful techniques reported for the analysis of PAHs are generally based either on capillary gas chromatography (GC) with mass spectrometric (MS) identification[1–3], or high-performance liquid chromatography (HPLC) using selective UV absorption[4–6], or fluorescence emission[7–11]. Capillary GC gives very efficient separations of PAH mixtures but has a limited selectivity with respect to mixed geometric isomers. Similarly MS has not yet been successfully applied to the identification of these isomers and this is a critical disadvantage in

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view of the dependence of tumorigenicity on molecular shape rather than size. Recent literature on this subject suggests that HPLC is fast becoming the preferred technique particularly where a relatively quick and reliable method is required for the measurement of a limited number of PAH components[12,13]. Natural mixtures of PAHs contain hundreds of compounds but most of these are only present in minute concentrations. However a few of these compounds can be classified as major components where they are present in concentrations at least an order greater than the minor components. For environmental purposes it is usually only necessary to measure the major components and so an important feature of a suitable method must be the appropriate selectivity to avoid interference from the minor components. This objective is most easily accomplished using fluorescence emission spectroscopy in conjunction with selective liquid chromatography.

This paper examines two fundamentally different aspects of selectivity, namely, selective retention, in which several chromatographic systems are successively applied to produce a high level of component separation, and selective detection, in which mixed components not separated by the earlier stages are subjected to conditions which will only respond to each component of the mixture in turn.

We have previously described the selective properties of HPLC using microparticulate silica gel combined with selective UV detection, and also of bentone-selective thin-layer chromatography or BS-TLC[6]. The properties of three different retentive systems have now been investigated in relation to the separation of PAHs, *viz.*, preparative-scale columns packed with the partially alkylated cross-linked dextran, Sephadex LH-20® (Pharmacia, Uppsala, Sweden), columns packed with microparticulate silica gel Partisil 5® (Whatman, Maidstone, Great Britain) and finally the use of the organo-clay Bentone 38® (dimethyldioctadecylammonium hectorite) (Baroid Division, N.L. Industries, Houston, TX,U.S.A.) as an adsorbent in two novel modes of TLC to produce rapid high-performance separations.

Fluorescence spectroscopy is employed for the detection and identification of PAHs because of its high sensitivity and selectivity, and the dependence of these properties on the excitation and emission wavelengths.

EXPERIMENTAL

A natural mixture of PAHs was obtained from a high-temperature coal tar pitch by exhaustive extraction with cyclohexane. The extract was washed with 10% sulphuric acid to remove basic material and the cyclohexane distilled off in a rotary evaporator under reduced pressure.

Preliminary separation by Sephadex LH-20

Preparative-scale chromatography of the cyclohexane-soluble material was carried out on a 1 m × 1.5 cm I.D. glass column (Corning, Staffs, Great Britain) packed with 60 g Sephadex LH-20 (Pharmacia), which had been left to swell in UltraR grade isopropanol (Hopkin & Williams, Romford, Great Britain) for 3 days. The isopropanol mobile phase was pumped through the column by means of a peristaltic pump at a flow-rate of 4–8 ml/h. The low solubility of PAHs in isopropanol precluded the use of this solvent to dissolve the pitch extract for application to the column and so 1 g of the extract was digested in 20 ml isopropanol to which several

grammes of Sephadex LH-20 had been added. The sample was absorbed almost entirely by the Sephadex which was then added to the previously packed column as an additional few centimetres of packed length. This proved to be a very efficient method of sample application. Fractions were collected hourly by means of an automatic fraction collector until a total of 169 fractions had been accumulated. After a preliminary examination by HPLC with UV detection these were bulked to 24 fractions, the solvent removed in the rotary evaporator, and each residue redissolved in a small volume of *n*-hexane.

High-performance liquid chromatography

Each of the bulked Sephadex fractions were examined by HPLC using a 25 cm × 4 mm I.D. column packed with 5-μm Partisil (Whatman). Aromatics-free *n*-hexane was used as the mobile phase, operated at a flow-rate of 2 ml/min with a constant-pressure HPLC pump (Jobling J.L911, Staffs, Great Britain). A grating ratio-recording spectrofluorimeter (Baird-Atomic SFR 100) was employed to monitor the eluted components via a quartz capillary flow cell. The system could be operated either in a continuous mode using appropriately selected but fixed excitation and emission wavelengths, or on a "stop–start" basis whereby the mobile phase was turned off by means of a valve situated just before the column, and the excitation and emission spectra of the material in the flow cell scanned. As many as 10–15 stop–start operations could be conveniently carried out during a single run without appreciable losses in resolution. For most purposes a sample volume of 5 μl was sufficient but in several cases sample volumes of up to 50 μl were employed with only slight losses in resolution. In all cases the addition of the sample to the HPLC column was made by microlitre syringe after turning off the flow of mobile phase.

Bentone-selective thin-layer chromatography

Two different modes of BS-TLC have been developed and applied to the separation of PAHs. For general separations of complex mixtures and for the rapid determination of benzo[*a*]pyrene, 10 × 20 cm glass plates were coated with a 0.01 mm layer of Bentone 38 slurried in methanol and using a Camag manual plate-coating machine. The freshly coated plates were allowed to dry in air at ambient temperature for 30 min and then in an oven at 140°C for 1 h. Before use the Bentone 38 was washed with methanol to remove free quaternary base halide. The plates were loaded with 0.1–1.0 μl of sample and developed in acetone–methanol (50:50) to a solvent migration distance of 10 cm, dried at ambient temperature and examined under UV light. Zones of interest were removed from the plate simply by touching the appropriate area with the end of a glass rod which had been ground flat, and then immersing the rod in a small volume of toluene. The very small inherent particle size of the organo-clay, and its small film thickness on the plate enabled the adsorbent to adhere quantitatively to the glass rod, but was then easily dispersed in toluene in which Bentone 38 forms a stable translucent gel. The resulting solution was transferred to a microcell for direct fluorescence measurements without the need for separate extraction and filtration stages.

BS-TLC was also applied to fractions taken from the HPLC column when spectroscopic measurements indicated the presence of two or more co-eluting components but having insufficient differences in their spectral properties to allow their

separate examination. For this purpose, 76 × 26 mm glass microscope slides were used, coated with a 0.01-mm layer of Bentone 38 and HPLC fractions were applied after concentrating them to a few microlitres. These microslide plates were developed in small screw-topped bottles containing a few millilitres of methanol–acetone mixture and developed to a solvent migration distance of only 5 cm with a development time averaging about 5 min. The plates were then dried and scanned automatically in the spectrofluorimeter by means of a simple modification which enabled the slide to be driven at a constant rate through the excitation beam. Fluorescence spectra of the separate zones were subsequently obtained by stopping the plate scan at the appropriate points and scanning the spectrum after adjusting the excitation and emission wavelengths as necessary. In practice, since each HPLC run can produce a number of such fractions, it was convenient to run a number of the BS-TLC plates simultaneously in separate bottles, with a considerable overall saving in time.

RESULTS AND DISCUSSION

Selectivity of PAH retention behaviour

Fig. 1 shows the relationship between molecular mass and log elution time for a number of PAHs on the Sephadex LH-20 preparative-scale column. This material is normally employed as a packing for lyophilic gel-permeation chromatography but, with PAHs, an adsorption–elution behaviour is obtained in polar solvents such as isopropanol. According to Streuli this behaviour is a function of the π-electron interaction with the adsorbent surface in which a Lewis acid-base pair is formed. This leads to a degree of selectivity depending on the resonance energy of the PAH molecule[14]. Fig. 1 demonstrates this selectivity which is seen to be very significant for parent, alkyl and partially hydrogenated PAHs, and to a lesser extent for alternant

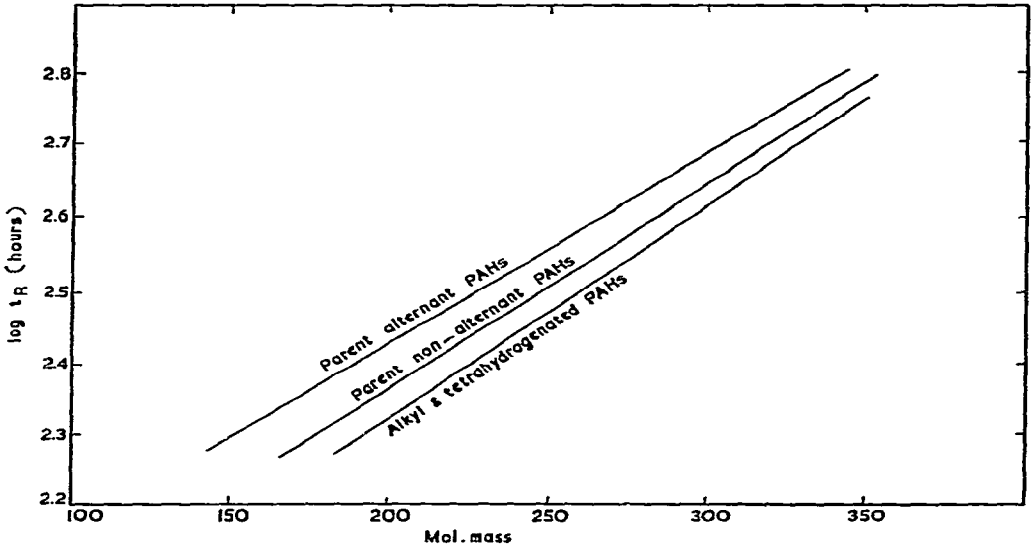

Fig. 1. Correlation of PAH retention properties on Sephadex LH-20 with molecular mass.

and non-alternant parent PAHs. Thus the bulked fractions from the Sephadex separation showed the presence of alkyl and partially hydrogenated derivatives of PAHs being eluted slightly in advance of their parents, and also of non-alternant PAHs before their alternant isomers. The retention properties of hydrogenated PAHs will obviously depend on the degree of hydrogenation, with increasing hydrogenation having the effect of decreasing the retention time. The experimental evidence suggests that with four additional hydrogen atoms per molecule the compound will elute from the Sephadex column with the alkyl derivatives having the same aromatic residue.

Somewhat different behaviour was observed on the Partisil 5 column, as shown in Fig. 2 in which log capacity ratio (k') is plotted against molecular mass. Again there is a general increase in retention with molecular mass but with a selectivity which is largely dependent on the "compactness" of the molecule. We have previously defined this compactness empirically as the degree of molecular condensation[6], given by

$$C_m = \frac{\text{number of C–C bonds shared by two rings}}{\text{number of carbon atoms in the molecule}} \times 100$$

and compounds giving similar values of C_m tend to fall on the same line.

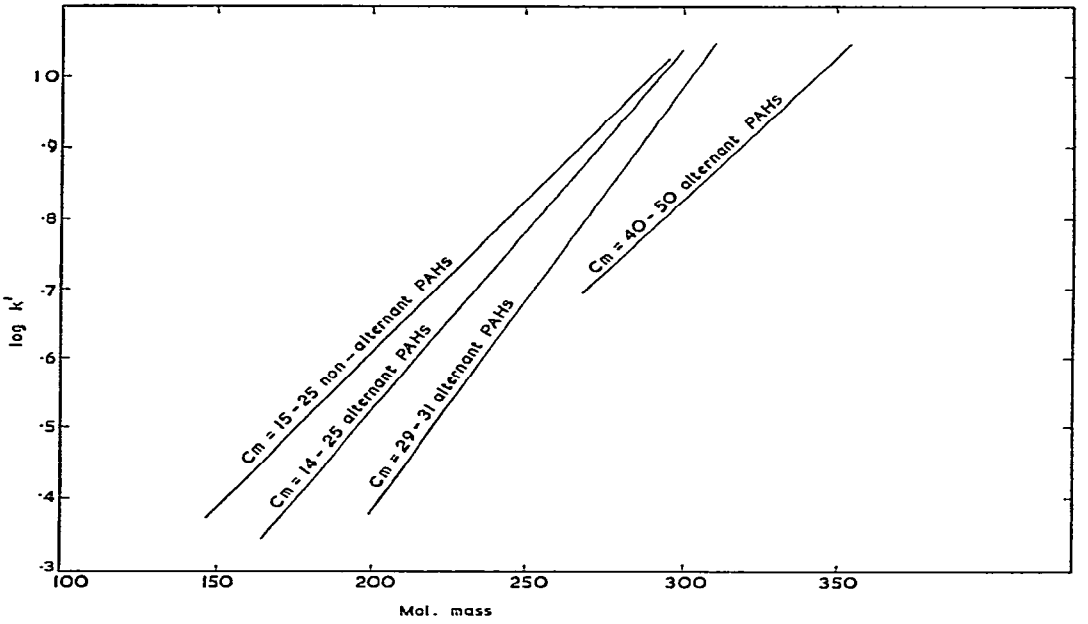

Fig. 2. Correlation of PAH retention properties on Partisil with molecular mass. Alkyl PAHs are eluted after their parent compounds. Partially hydrogenated PAHs are eluted before their parent PAHs but after the PAHs corresponding to the aromatic residue.

The combined selectivity on the two columns was of immense value in helping to characterise many of the PAHs in the pitch solvent extract. Thus, although non-alternant PAHs such as benzo- and dibenzo-fluoranthenes occur in earlier Sephadex fractions than their alternant isomers, the benzo- and dibenzopyrenes, they are more

strongly retained on the Partisil HPLC column. Alkyl PAHs behave similarly to non-alternant but partially hydrogenated PAHs elute earlier than their parent compounds on both Sephadex and Partisil, hence enabling the latter two types of compound to be differentiated. Fig. 3 shows an HPLC run carried out on the original sample before its separation by Sephadex LH-20. Detection was by UV absorbance at 300 nm as this gives a more accurate visual impression of the relative amounts of the different components than fluorescence emission, which is far more selective in character. This HPLC–UV run on the original sample was used as a reference separation with regard to the labelling of the peaks which are numbered consecutively. The identification of the major components was the result of earlier work previously described[6]. Fig. 4a–f shows the separations of selected fractions from the Sephadex run but using the spectrofluorimeter for detection. A comparison of the chromatograms demonstrates some of the selective features on the two columns. The fractions clearly increase generally in their average molecular mass but in fraction 4 the benzofluorenes (molecular mass 216) elute in the pyrene–fluoranthene-rich fraction (molecular mass 202), and in fraction 6 benzo[*k*]fluoranthene (molecular mass 252) appears with benz[*a*]anthracene and chrysene (molecular mass 228). One aspect of the Partisil selectivity is shown in the case of fraction 13 where there are wide separations between anthanthrene and benzo[*ghi*]perylene (molecular mass 276) and the group of $C_{22}H_{14}$

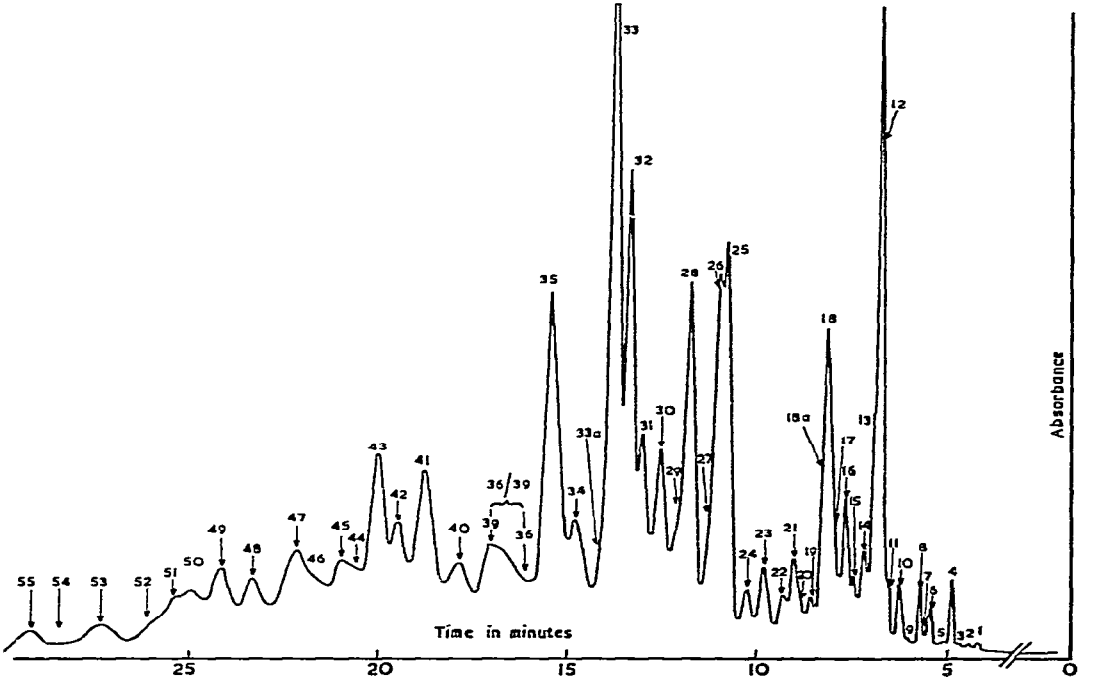

Fig. 3. Chromatogram of the cyclohexane extract of high-temperature pitch run on Partisil 5 after UV detection at 300 nm. Major peak identities: 12 = pyrene; 18 = fluoranthene; 25 = benz[*a*]anthracene; 26 = chrysene; 28 = benzo[*a*]pyrene; 30 = benzo[*e*]pyrene; 31 = anthanthrene; 32 = benzo[*k*]fluoranthene; 33 = benzo[*b*]fluoranthene + benzo[*j*]fluoranthene; 35 = indeno[123, *cd*]-pyrene + coronene; 41 = dibenz[*ai*]anthracene; 43 = dibenz[*ah*]anthracene; 47 = dibenzo[*ah*]-pyrene + dibenzo[*ai*]pyrene; 48 = dibenzo[*ae*]pyrene.

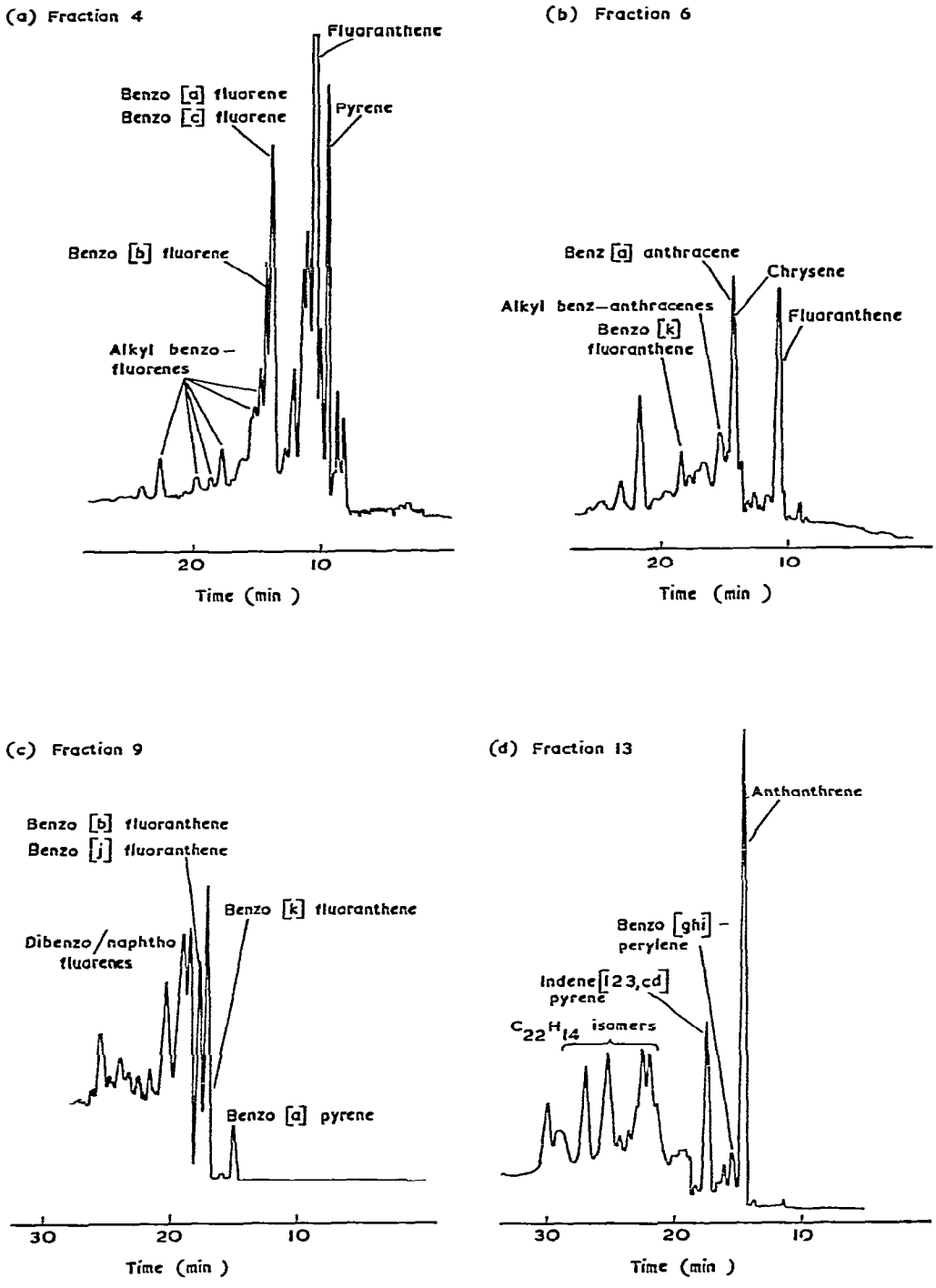

Fig. 4.

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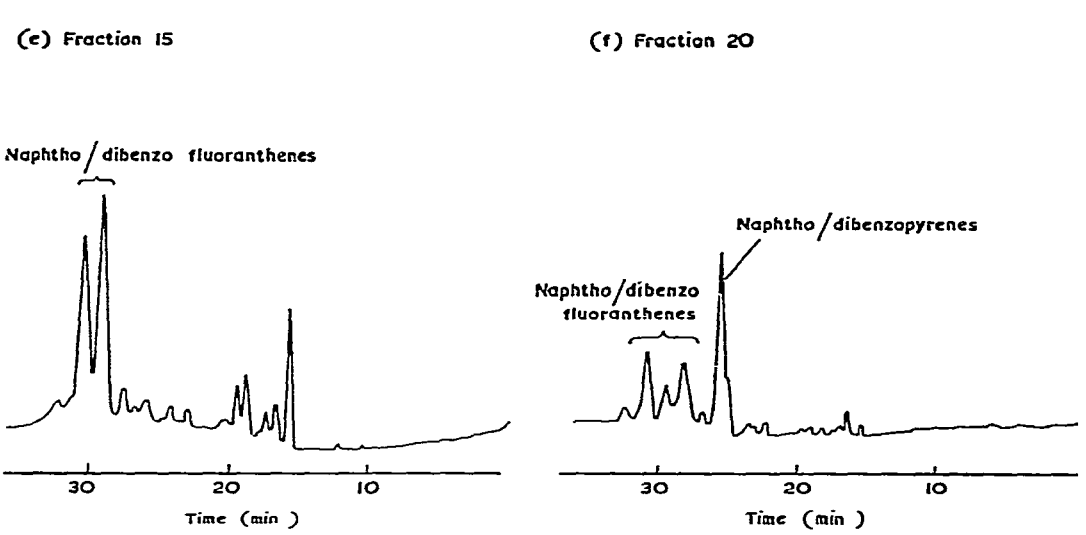


Fig. 4. Chromatograms of selected Sephadex LH-20 fractions on Partisil 5. Conditions: *n*-hexane mobile phase at 2 ml/min; spectrofluorimetric monitoring at excitation wavelength 300 nm and zero order emission.

isomers (molecular mass 278). Finally fractions 15 and 20 contrast the different selectivities of Sephadex LH-20 and Partisil toward the isomeric dibenzo- and naphthofluoranthenes and the dibenzo- and naphthopyrenes. Fraction 15 contains mainly the former non-alternant compounds, whilst the later fraction 20 contains principally the alternant isomers (which elute earlier from the Partisil column than the non-alternant isomers). Some of the hydrocarbon types identified by retention characteristics alone are given in Table I. Many of these were later confirmed by fluorescence spectroscopy as indicated in the last column.

Choice of column for HPLC separations of PAHs

The majority of published work in recent years recommends the use of bonded-phase columns for the separation of PAHs. Ogan and Katz[15] have recently published a study of various commercial C_{18} bonded phase materials with regard to their retention properties in the analysis of PAHs and Table II shows some of this data as measured from the published graphical results. These are compared with our results using an adsorption column packed with Partisil 5. For the bonded-phase columns the mobile phase comprised a mixture of acetonitrile and water (80:20), and for the adsorption column, aromatics-free *n*-hexane. The small scale of the published graphs prevented the measurement of k' values to better than 0.5 units but this is sufficiently accurate to indicate the retention behaviour and the degree of separation. Obviously there are considerable variations in retention behaviour depending on such factors as surface coverage of C_{18} groups and the proportion of residual unreacted silanol groups on the silica surface. Generally the selectivity is poor on the bonded phase columns except for column 5 which separated all the compounds reported but did not include data for the important isomers, perylene and benzo[*j*]fluoranthene. In contrast, the Partisil column separated all the compounds selectively with the exception of benzo-

TABLE I

THE USE OF THE COMBINED RETENTION PROPERTIES OF PAHs ON SEPHADEX LH-20 AND PARTISIL 5 TO INDICATE THE CHARACTER OR IDENTITY OF HPLC PEAKS

Fraction Nos. with maximal concentration	*Peak Nos. (see Fig. 3)*	*Suspected identity or character*	*Confirmed identity by fluorescence spectroscopy*
4	14	Alkyl pyrene	Methyl pyrene
4	15	Alkyl pyrene	2-Methyl pyrene
4	16	Alkyl pyrene	1-Methyl pyrene
4	18	Fluoranthene	Fluoranthene
4	20–23	Alkyl fluoranthenes	Alkyl fluoranthenes
4, 5	12	Pyrene	Pyrene
5	26–35	Alkyl benz[*a*]anthracenes	Alkyl benz[*a*]anthracenes
6	25	Benz[*a*]anthracene	Benz[*a*]anthracene
7	33–41	Alkyl benzofluoranthenes	Alkyl benzo[*a*]fluoranthenes
7, 8	20	Partially hydrogenated compound	Tetrahydrobenzo[*a*]pyrene
8	34–47	Alkyl benzofluoranthenes	Alkyl benzo[*b*]fluoranthenes
9	23	Partially hydrogenated compound	Dihydrobenzo[*a*]pyrene
9	28	Benzo[*a*]pyrene	Benzo[*a*]pyrene
9	31	Alkyl benzo[*a*]pyrene	Alkyl benzo[*a*]pyrene
9	32	Benzo[*k*]fluoranthene	Benzo[*k*]fluoranthene
9	33	Benzo[*b* or *j*]fluoranthene	Benzo[*b*]fluoranthene
9	35–39	Alkyl benzofluoranthenes	Alkyl benzo[*j*]fluoranthenes
9	40–47	Dibenzo- and naphthofluorenes	Dibenzo- and naphthofluorenes
12	35	Indeno[123, *cd*]pyrene	Indeno[123, *cd*]pyrene
12	36–40	$C_{22}H_{12}$ non-alternant PAHs	Probable C_{22} dibenzofluoranthenes
13	40–45	$C_{22}H_{14}$ alternant PAHs	Mainly $C_{22}H_{14}$ PAH (see Table III)
16	49–51	$C_{24}H_{14}$ non-alternant PAHs	Dibenzo–naphtho fluoranthenes
21	46	$C_{24}H_{14}$ alternant PAH	Naphtho[23, *a*]pyrene
21	47	$C_{24}H_{14}$ alternant PAH	Dibenzo[*ah*]pyrene Dibenzo[*ai*]pyrene Naphtho[12, *a*]pyrene
21	48	$C_{24}H_{14}$ alternant PAH	Dibenzo[*ae*]pyrene

TABLE II

COMPARISON OF PAH RETENTION CHARACTERISTICS ON BONDED PHASE AND SILICA GEL ADSORPTION COLUMNS

Columns: 1, μBondapak (Waters Assoc., Milford, MA, U.S.A.); 2, Zorbax ODS (DuPont, Wilmington, DE, U.S.A.); 3, Partisil 10 ODS (Whatman); 4, LiChrosorb RP-18 (E. Merck, Darmstadt, G.F.R.); 5, HC-ODS (Separation Group); 6, balanced-density packed using Whatmans Partisil 5. *k'* values were calculated from data published by Ogan and Katz[15].

Compound	*Molecular formula*	*k' values of bonded-phase columns*					*Partisil 5 (containing 3.5% water)*
		1	*2*	*3*	*4*	*5*	*6*
Benz[*a*]anthracene	$C_{18}H_{12}$	2.5	8.0	8.0	6.0	2.5	8.7
Chrysene	$C_{18}H_{12}$	2.5	8.0	8.0	6.0	3.0	9.0
Benzo[*e*]pyrene	$C_{20}H_{12}$	4.0	12.5	13.5	10.0	4.0	10.1
Benzo[*a*]pyrene	$C_{20}H_{12}$	4.0	12.0	16.0	13.0	8.0	9.5
Perylene	$C_{20}H_{12}$						10.5
Benzo[*b*]fluoranthene	$C_{20}H_{12}$	4.0	12.0	13.0	10.0	4.5	11.4
Benzo[*k*]fluoranthene	$C_{20}H_{12}$	4.0	12.5	13.5	11.0	6.5	11.1
Benzo[*j*]fluoranthene	$C_{20}H_{12}$						11.4
Benzo[*ghi*]perylene	$C_{22}H_{12}$	5.0	22.0	28.0	20.0	13.0	10.7
Indeno[123, *cd*]pyrene	$C_{22}H_{12}$	5.0	21.5	24.5	20.0	16.0	10.5

[*b*]fluoranthene and benzo[*j*]fluoranthene. We have also found little variation in this behaviour using different batches of silica from different manufacturers but all adjusted to the same water content. Thus we feel that the separation on silica gel is a fundamental property of the silica surface, and consequently can be standardized for analytical purposes.

Effect of acetone addition to the mobile phase

The selectivity of Partisil columns towards PAHs were found to be critically affected by the presence of small concentrations of acetone in the *n*-hexane mobile phase. Fig. 5 shows this effect for a number of the more important compounds, all of which elute between fluoranthene and indeno[123,*cd*]pyrene. The retention characteristics were measured in terms of a "selectivity index" (S_I) which we define as

$$S_I = 100 + 100 \frac{t_R \text{ (component)} - t_R \text{ (fluoranthene)}}{t_R \text{ (indeno[123, } cd\text{]pyrene)} - t_R \text{ (fluoranthene)}}$$

where t_R is the retention time measured from injection.

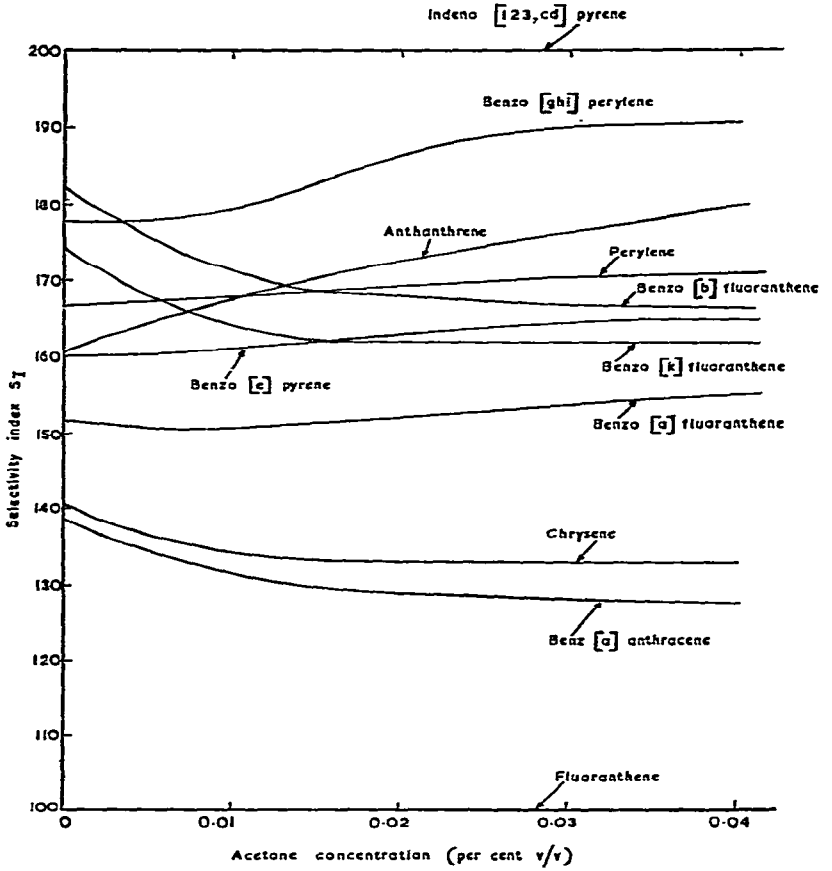


Fig. 5. Effect of acetone concentration on PAH retention characteristics. Conditions: column 25 cm × 4 mm I.D. packed with Partisil 5; mobile phase *n*-hexane at 2 ml/min.

Thus the components eluting between fluoranthene and indeno[123,*cd*]pyrene are placed on a scale of 100–200 defined by the difference in retention time between the two marker compounds and divided into 100 equal units. This method nullifies the effect of small flow variations between injections and also obviates the difficulty in this form of HPLC of measuring the column hold-up volume as S_I is independent of this quantity. S_I is of course reminiscent of the Kováts retention index in GC and has been found to be a convenient and precise method of expressing the retention characteristics of PAHs in a way that enables column performance to be standardized at a particular level of selectivity. It is also a simple algebraic function of the conventional separation factor of the three components involved in the calculation and so it is a true measure of thermodynamic selectivity. Fig. 5 shows selective effects for the non-alternant benzofluoranthenes, for benzo[*ghi*]perylene, and for benz[*a*]-anthracene and chrysene. In several instances these effects are sufficient to cause reversals in the elution order, as with anthanthrene–perylene–benzo[*b*]fluoranthene, and with benzo[*e*]pyrene–benzo[*k*]fluoranthene. For most purposes an acetone concentration of 0.01% (v/v) will give an optimum separation of the important compounds, particularly when combined with selective fluorescence emission.

Spectrofluorimetric monitoring of PAHs

All PAH fluoresce when exposed to UV radiation by virtue of their π-electron configurations which are excited to higher energy singlet states in solution. This excess energy is subsequently released partly by vibrational relaxation through thermal transfer to the solvent environment and partly by the emission of a photon at a lower energy than the absorbed photon. Consequently most PAHs fluoresce in the visible region of the spectrum.

The fluorescence characteristics of PAHs are widely utilized for their detection and identification principally because the method has a high inherent sensitivity and selectivity. In contrast to UV absorption spectroscopy, the emission spectrum of each component of a complex mixture can often be obtained separately by selecting appropriate excitation wavelengths where only the component of interest absorbs energy. Thus Fig. 6 shows emission spectra for an HPLC peak obtained during the current studies on Sephadex fractions. Spectrum a shows a combined spectrum for all the components, and b and c show selective runs showing the individual compounds benz[*a*]anthracene and chrysene which were not sufficiently separated by this particular column.

Thus we see that by running the chromatogram at an excitation wavelength (λ_{ex}) of 285 nm and an emission wavelength (λ_{em}) of 386 nm only benz[*a*]anthracene is detected in peak No. 25 hence enabling its quantitative determination without interference by co-eluting components.

Two of the benzofluoranthene isomers also co-elute from most columns, there being no previously published determinations of benzo[*b*]fluoranthene and benzo[*j*]-fluoranthene separately. We have successfully determined the [*b*] isomer at λ_{ex} 350 nm and λ_{em} 500 nm, and the [*j*] isomer at λ_{ex} 385 nm and λ_{em} 550 nm.

Characterization of PAH types

According to Sawicki *et al.*[16] alternant PAHs can be differentiated from non-alternant PAHs by their differing fluorescence quenching characteristics in the presence

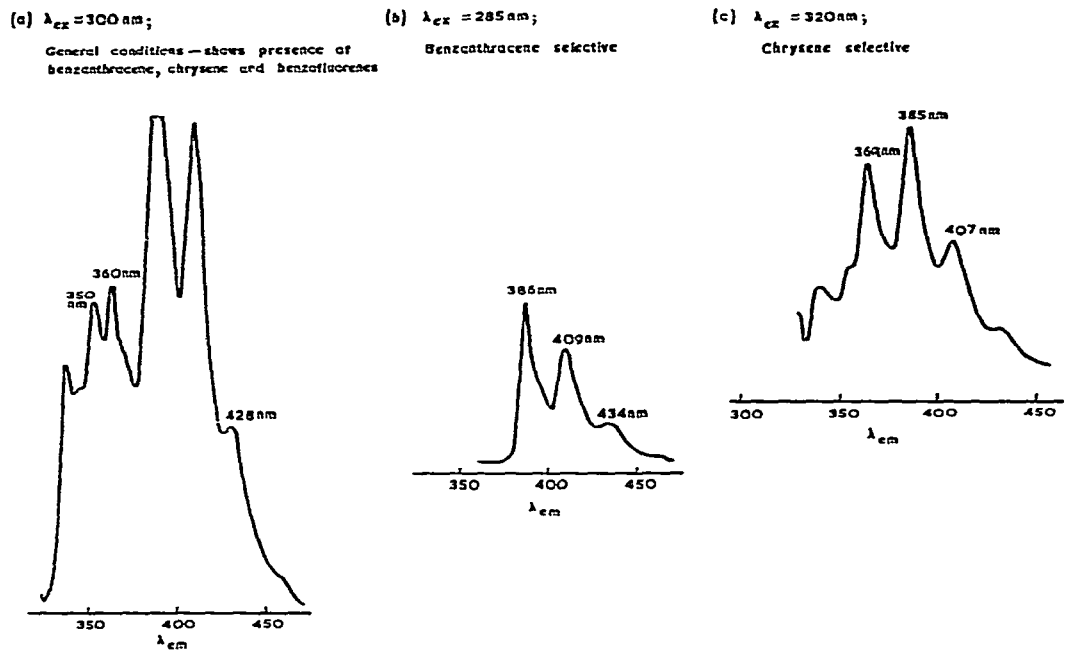


Fig. 6. Selective emission scans of peak 25 from HPLC of Sephadex fraction No. 6 showing the presence of benz[*a*]anthracene and chrysene.

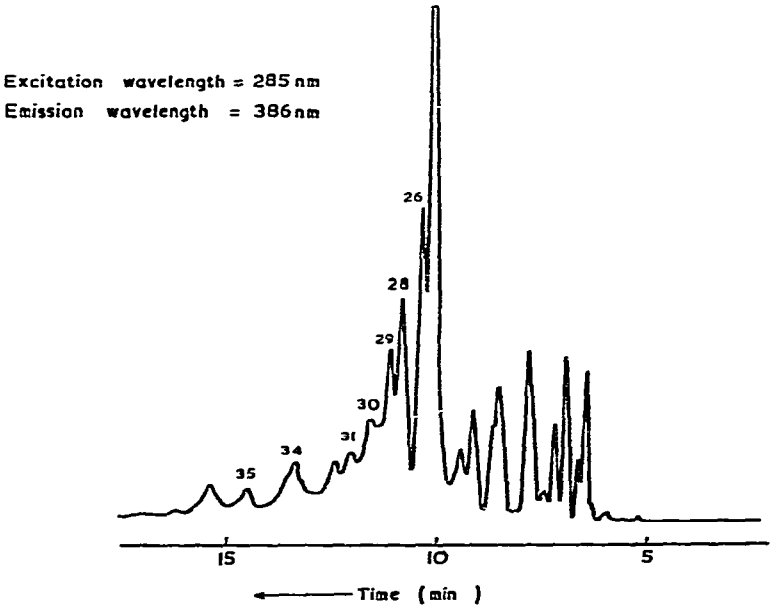


Fig. 7. Chromatogram of Sephadex fraction No. 5 under selective conditions for the detection of benz[*a*]anthracene derivatives.

of nitromethane. Zander and co-workers[17,18] have extended this work to a study of various charge transfer complexing agents and have shown that "quenchofluorimetry" can be applied to complex mixtures of the two hydrocarbon types quantitatively. Our investigations have not been concerned with such a generalised classification of alternant and non-alternant systems because the high level of separation afforded by the chromatographic systems and their combined retention properties offer a strong indication of the hydrocarbon type. When considered in conjunction with fluorescence characteristics the classification of a large number of the hitherto unidentified components becomes possible. For instance alkyl derivatives of PAHs gave emission spectra which were very similar to those for the parent compounds, considerably simplifying their identification. Fig. 7 shows a chromatogram of Sephadex fraction 5 (see Table I) run selectively at λ_{ex} 285 nm and λ_{em} 386 nm. Although these conditions selectively respond to benz[*a*]anthracene and its alkyl derivatives they are not specific to these compounds as other compounds which also absorb and emit energy at these wavelengths will also give a response. Confirmation of component identity was therefore made by scanning the full emission spectrum for each peak using the stop–start procedure. Fig. 8 shows these scans for those peaks characterized as alkyl benz[*a*]anthracenes.

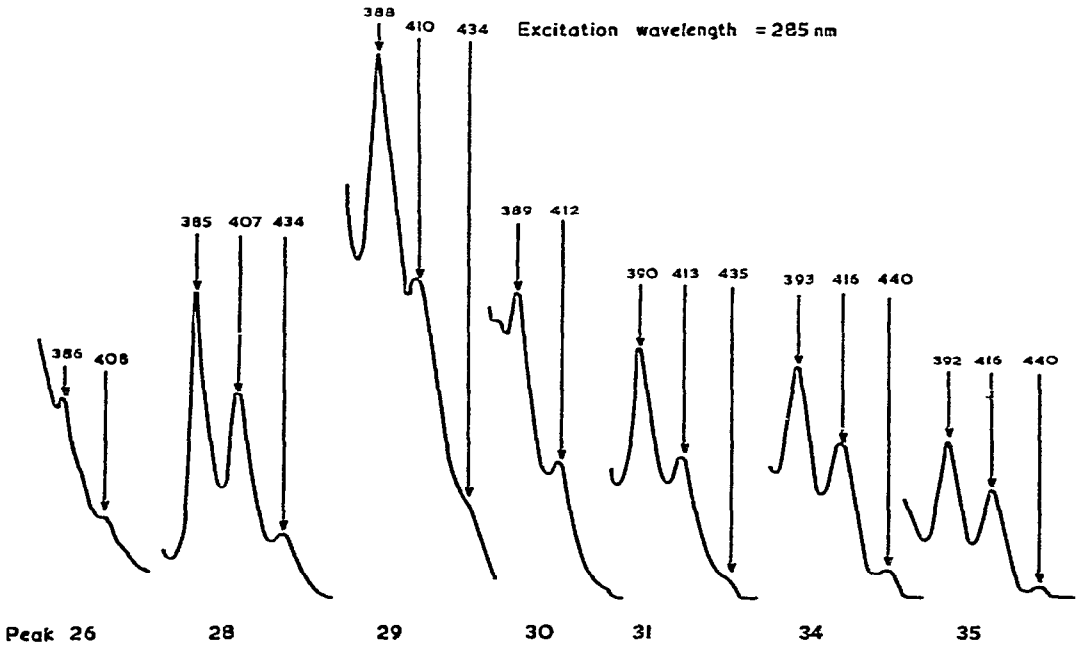


Fig. 8. Fluorescence emission spectra of HPLC peaks obtained by stop–start run of chromatogram shown in Fig. 7.

Partially hydrogenated PAHs were also characterized by their fluorescence properties, particularly when these were considered in conjunction with their retention behaviour on Sephadex LH-20 and Partisil 5. An example of this is shown in Fig. 9 which is a comparison between a known pyrene derivative (1-ethyl pyrene) and a

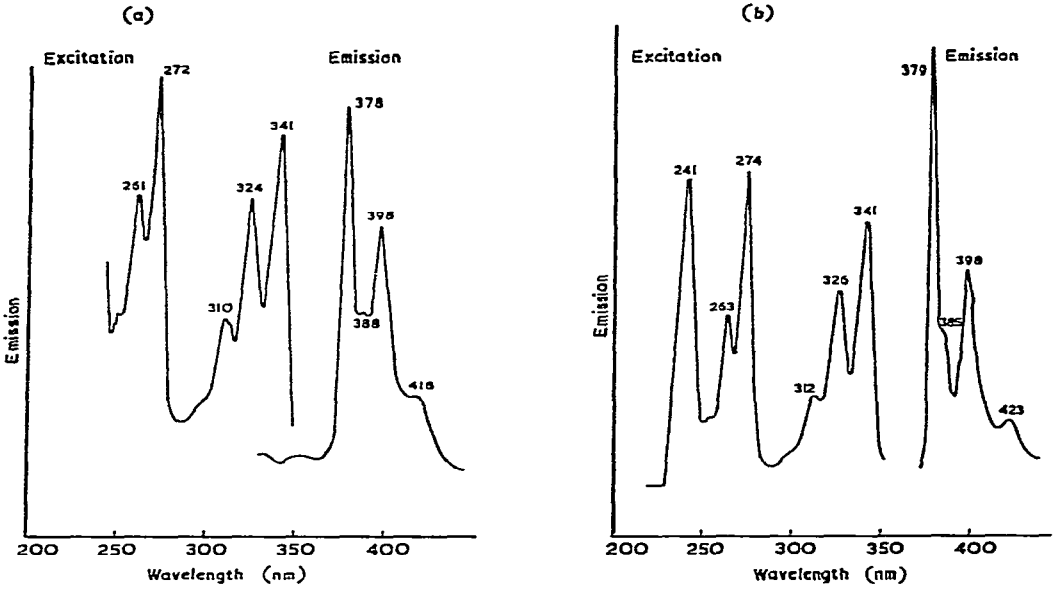
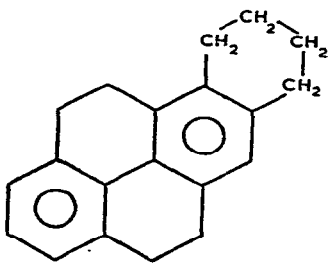


Fig. 9. Characterization of pyrene-type component. (a), 1-Ethyl pyrene; (b), fraction 7, peak 18.

component of Sephadex fraction No. 7. Alkyl pyrenes, however, elute in the region of Sephadex fraction No. 4, and the retention time on Partisil is much higher than could be accounted for by this type of component. Retention properties on both stationary phases are appropriate for a partially hydrogenated five-ring compound with a "pyrene-like" aromatic residue. Comparison of the excitation spectrum with published UV absorption spectra suggested that the identify of the component was 1,2,3,4-tetrahydrobenzo[*a*]pyrene, *viz.*:

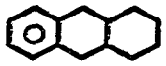
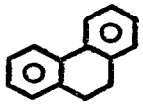
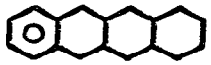
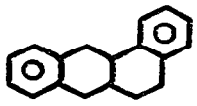
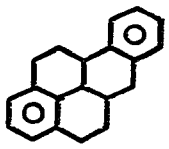
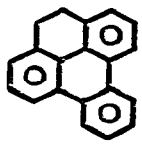
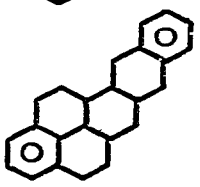
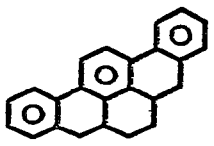
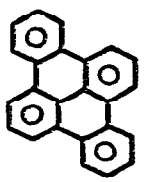


Relationship between spectral characteristics and structure

There are several spectral characteristics which are qualitatively related to structure and which have been utilized in the identification of geometric isomers. The number of separate π-electron sextets is an indication of the extent of electron delocation in the hydrocarbon molecule; the greater the number of sextets the greater is the stability of the molecule, and so the higher is the emission energy (shorter wavelength). Table III gives some examples of the effect of structure on fluorescence emission properties. Comparing the compounds naphthalene, anthracene and naph-

TABLE III

RELATIONSHIP BETWEEN PAH STRUCTURE AND FLUORESCENCE EMISSION
S = Shoulder.

Compound	*Structure*	*No. sextets*	λ_{em} *(nm)*
Naphthalene		1	323, 335, 347 (s)
Anthracene		1	382, 398, 422, 448
Phenanthrene		2	350, 357, 366, 377
Naphthacene		1	471, 504
Benz[*a*]anthracene		2	386, 408, 432
Benzo[*a*]pyrene		2	405, 428, 455
Benzo[*e*]pyrene		3	389, 398, 410
Naphtho[2:3, *a*]pyrene		2	460, 491, 526
Dibenzo[*ai*]pyrene		3	433, 448, 460
Dibenzo[*e*, 1]pyrene		4	374, 386, 394, 406

thacene in which the benzene rings are linearly annellated, thus containing only one completed sextet, the emission band increases in wavelength in a regular manner. Thus the effect of the increasing molecular mass is to increase the extent of electron delocation, decreasing the molecular orbital energy which facilitates excitation and emission at lower energies, or high wavelengths. Comparing linearly annellated PAHs with their angularly annellated isomers we find that the addition of another complete sextet increases the stability of the molecule, resulting in a hypsochromic shift of the emission band. The effect of the number of complete sextets on the emission spectra of isomers is also demonstrated by comparing benzo[*a*]pyrene with benzo[*e*]-pyrene and also between the $C_{24}H_{14}$ isomers. In fact the presence of naphtho[2:3,*a*]-pyrene (see Table I) was initially suggested by the predicted effect on the emission spectrum of the number of completed sextets.

Further separation of HPLC peaks by BS-TLC

The microslide BS-TLC technique was applied to HPLC peaks corresponding to $C_{22}H_{14}$ isomers present in Sephadex fraction No. 13 (see Fig. 4d) where there are twelve possible isomers with this molecular weight, eluting over a small retention region. Fig. 10 shows a fluorescence emission scan of a BS-TLC microslide plate separation of peak 43 (see Fig. 3) in a search for the dibenz[*ah*]anthracene isomer.

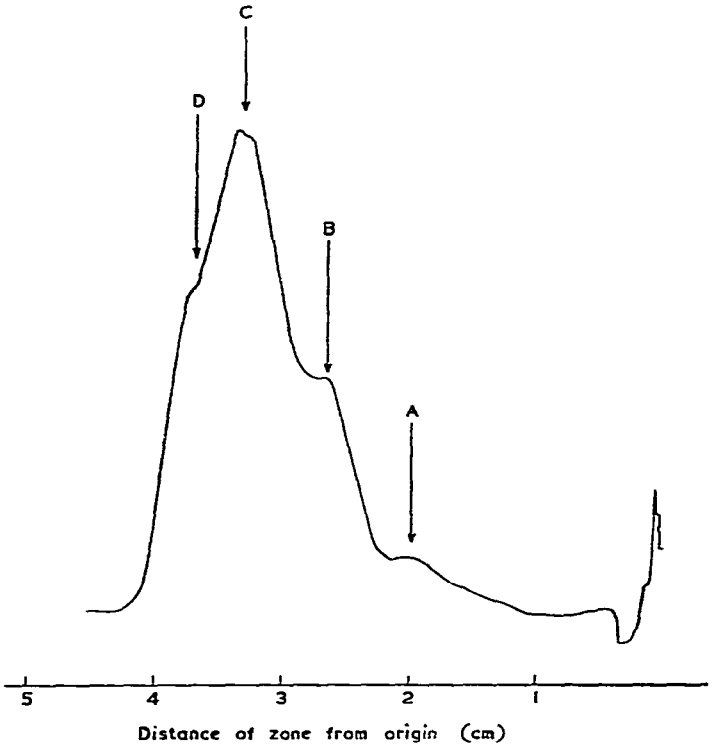


Fig. 10. Automatic scan of BS-TLC microslide plate showing separation of peak No. 43 from Sephadex fraction No. 13.

The excitation and emission spectra for zone D, scanned *in situ* on the plate, and comparison spectra for an authentic specimen run under identical conditions are shown in Fig. 11, and confirm the presence of this compound. Quantitative measurements indicated that the concentration of dibenz[*ah*]anthracene in the original sample was some two orders of magnitude lower than the major PAH components, hence demonstrating the high sensitivity of the technique. Table IV summarises the results of applying this scanning BS-TLC method to HPLC peaks corresponding to $C_{22}H_{14}$ isomers. Since in many cases the "TLC position" does not necessarily coincide with the centre of a solute zone, the use of R_F data is invalid. Thus the labelled positions refer to consecutive zones on the plate, and in some cases correspond to different positions on the same apparent peak. Of the twelve possible $C_{22}H_{14}$ isomers, only pentacene has a single complete sextet and, as indicated earlier, will emit well into the visible part of the spectrum. However, selective fluorescence runs have failed to detect this compound, presumably because of its extremely low solubility in most solvents. Many of the other components consisted either of alkyl $C_{22}H_{14}$ isomers or non-alternant $C_{22}H_{12}$ isomers which were characterized from their fluoranthene-like fluorescence emission spectra.

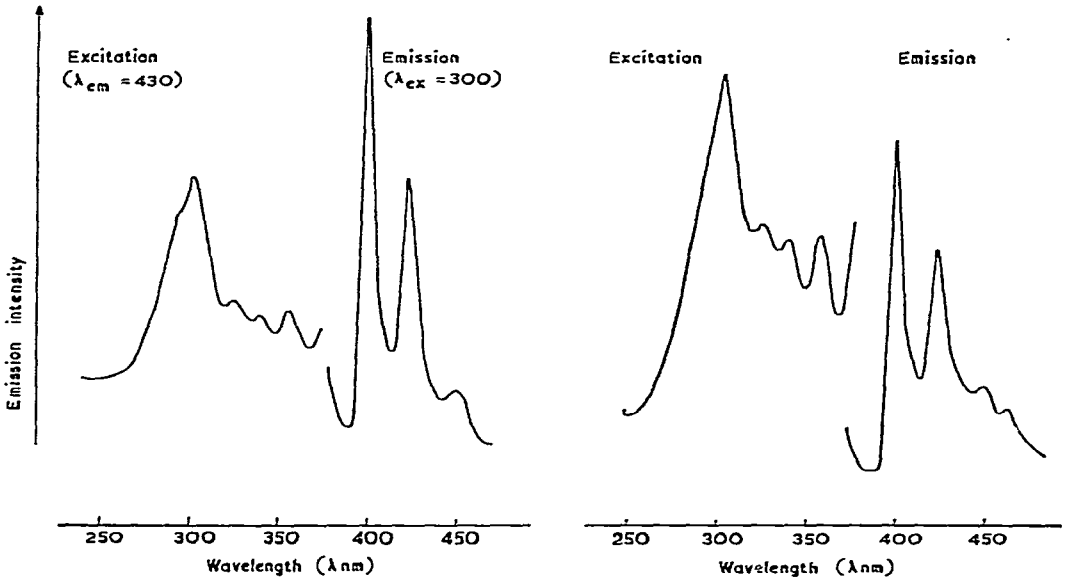


Fig. 11. Excitation and emission spectra of BS-TLC zone D from HPLC peak 43 confirming presence of dibenz[*ah*]anthracene. Left: zone D. Right: dibenz[*ah*]anthracene after BS-TLC; conditions as zone D.

Rapid determination of benzo[a]pyrene by BS-TLC

The direct BS-TLC–spectrofluorimetric technique in which separated zones are collected on a glass rod, dispersed in toluene and measured by fluorescence emission, was applied to the rapid determination of benzo[*a*]pyrene in environmental samples. Fig. 12 shows the emission spectrum of the benzo[*a*]pyrene zone from a sample of atmospheric particulates compared to a parallel run reference sample of

TABLE IV

APPLICATION OF BS-TLC WITH FLUORESCENCE SCANNING TO HPLC PEAKS 40–49 FROM SEPHADEX FRACTION 13

s = Shoulder.

Peak No.	*TLC position*	*Principle emission peaks, wavelength (nm)*	*Comments and possible identity*
40	A	No distinctive features	
	B	394 only	Unidentified
	C	400, 420	Picene
	D	393 only	Similar to B
	E	396, 420	$C_{22}H_{14}$ isomer
41	A	408, 424, 462	$C_{22}H_{12}$ non-alternant PAH
	B	396, 406, 418, 428	dibenz[*ai*]anthracene
	C	No distinctive features	
42	A	No distinctive features	
	B	394 only	Similar to 40B
	C	400–420 diffuse	Mixture $C_{22}H_{14}$ isomers
	D	394, 422, 456, 486	Mixture $C_{22}H_{14}$ isomers and
	E		benzo[*a*]naphthacene
42a	A	402, 413, 424, 440	Mixture $C_{22}H_{14}$ isomers
	B	402, 410, 422, 433	Mixture $C_{22}H_{14}$ isomer
	C		
	D	400, 420, 450	Benzo[*b*]chrysene
	E		
43	A	406, 430, diffuse	$C_{22}H_{14}$ isomer
(see Figs. 2–4)	B	396, 400, 418(s) diffuse	Unidentified
	C	400, 424, 450	Benzo[*b*]chrysene (from 42a)
	D	400, 423, 450	Dibenz[*ah*]anthracene
44	A	400, 422 (very small)	Alkyl $C_{22}H_{14}$ isomer
	B	402, 409(s), 423	Alkyl dibenz[*ah*]anthracene
	C	400, 408(s), 423	Alkyl dibenz[*ah*]anthracene
		450 diffuse	Non-alternant PAHs
	D	400, 408(s), 432	Alkyl dibenz[*ah*]anthracene
45	A	400, 423	Alkyl dibenz[*ah*]anthracene
	B	400, 423, 450	Alkyl $C_{22}H_{14}$ isomer
	C	401, 411, 424, 450	Alkyl $C_{22}H_{14}$ isomer
	D	401, 409, 424, 450	Alkyl $C_{22}H_{14}$ isomer
47	A	402, 424, 435(s)	Alkyl $C_{22}H_{14}$ isomer
	B	401, 411, 424, 437(s)	Alkyl dibenz[*ah*]anthracene
	C	401, 411(s), 424, 450(s)	Alkyl dibenz[*ah*]anthracene
	D	402, 425, 450	Mixture of alkyl $C_{22}H_{14}$ isomer and partially hydrogenated $C_{24}H_{14}$ non-alternant PAHs
49	A	400–500 small peaks	Complex mixtures of trace components
	B	398, 406(s)	Alkyl $C_{22}H_{14}$ isomer
		460 very diffuse	Very similar to indeno[123, *cd*]-pyrene —probably a partially hydrogenated $C_{24}H_{14}$ non-alternant PAH

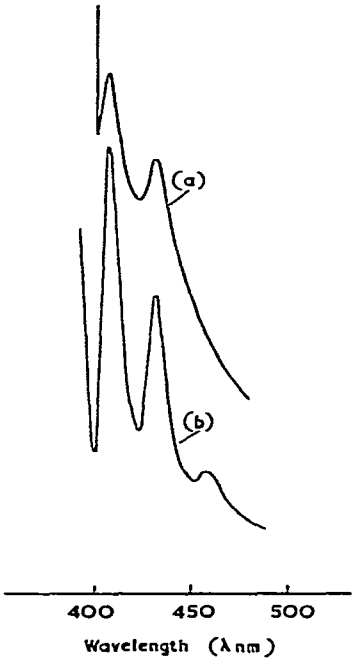


Fig. 12. Rapid determination of benzo[*a*]pyrene by BS-TLC. (a), Emission spectrum of BaP zone from atmospheric particulates; sensitivity, ×100; 1-μl aliquot from 50-μl concentrate of solvent extract; total benzo[*a*]pyrene in extract, 110 ng. (b), Spectrum from standard BaP solution subjected to identical treatment; sensitivity, ×3; 134 ng benzo[*a*]pyrene. λ_{ex} 380 nm.

pure material. This determination required a total analysis time of about 1 h including the time necessary for ultrasonic extraction of the filter in cyclohexane and concentration of the extract.

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