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USE OF CHEMICALLY MODIFIED MICROPARTICULATE SILICA AND SELECTIVE FLUORIMETRIC DETECTION FOR THE ANALYSIS OF POLYNUCLEAR HYDROCARBONS BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

B. B. WHEALS, C. G. VAUGHAN and M. J. WHITEHOUSE
Metropolitan Police Forensic Science Lab., 109 Lambeth Road, London S.E. 1 (Great Britain)
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SUMMARY

The preparation of a packing material for high-pressure liquid chromatography (HPLC) based on a microparticulate silica chemically bonded with octadecyltrichlorosilane is described. The performance of columns packed with this material in the separation of polynuclear hydrocarbons has been studied. The use of selective fluorimetric detection for the characterisation of polynuclear hydrocarbons in HPLC eluates is shown and the construction of an inexpensive detector detailed.

INTRODUCTION

The characterisation of complex mixtures of polynuclear hydrocarbons is a common analytical problem, and the chromatographic methods used to separate and identify these compounds have been the subject of review[1]. In a previous paper the use of high-pressure liquid chromatography (HPLC) on "reversed-phase" column systems as an aid to the separation of polynuclear hydrocarbons was described[2]. The present paper extends this area of work and describes improvements in chromatographic resolution achieved by the use of columns containing a chemically modified microparticulate silica. The use of fluorimetric detection confers appreciable selectivity on the analysis of mixtures of polynuclear hydrocarbons and studies linking HPLC and fluorimetry are described.

EXPERIMENTAL

Chemical modification of the silica

The column packing material used in these experiments was prepared by refluxing a small-particle-size porous silica (Partisil 5, of 7-μ average particle size, obtained from H. Reeve Angel, Maidstone, Great Britain) with 2 *N* HCl for 2 h. The silica was then washed with water until the washings were neutral, and finally washed several times with methanol. After drying overnight at 75°, 7 g of the silica was refluxed with 40 ml of *m*-xylene and 5 ml of octadecyltrichlorosilane for 2 h.

The chemically modified silica was washed several times with xylene, Soxhlet extracted for several hours with xylene, and then with methanol. The extracted material was dried at 75° overnight and was gently sieved before use.

Columns and packing procedure

Stainless-steel columns of 25 cm length and 6.35 mm O.D. (*i.e.* ¼ in.) and 4.9 mm I.D. were fitted with ¼-in. stainless-steel (Swagelok) nuts at each end. The bottom of the column was fitted with a 1/4- to 1/16-in. reducing (Swagelok) union blocked with a plug of porous PTFE (4 mm thick and of nominal 75-μ pore size, obtainable from Phase Separations, Queensferry, Flints., Great Britain). The column was packed with the modified silica using a balanced-density slurry technique[3] with 32% bromoform in chloroform as the suspending medium. After packing at *ca.* 5000–6000 p.s.i., a small portion of the silica was removed from the top of the column and a plug of porous PTFE inserted. Alternatively a disc of glass fibre filter paper, or cellulose acetate film (*e.g.*, Millipore filter), was placed on top of the packing with an overlaying 400-mesh stainless-steel disc to prevent syringe needle penetration.

Injector, pump and UV detector

The column was attached to a ball valve (Hoke miniature forged ball valve Type 7115 G 4 S, obtainable from Hoke International, New Barnet, Herts., Great Britain), modified by having a length of 1/16-in.-O.D. and 0.25-mm-I.D. stainless-steel tube silver-soldered into a hole drilled through the removable plug on the downstream side of the ball. This tube acts as a solvent inlet and converts the valve into an inexpensive stop-flow injector, which we have found to be capable of withstanding pressures of up to 6000 p.s.i. To aid central location of the injection needle on the top of the column a guide consisting of a short length of vertically mounted narrow-bore tubing was attached to the top of the valve. The soldered side arm of the modified valve was attached to a high-pressure pump (Waters Model No. 6000). The exit end of the column was coupled via a short length of microbore tubing to either a UV detector (Cecil Instruments, Model No. 212) or a fluorimetric detector, and in some cases was connected to both in sequence.

Fluorimetric detectors

Two detectors have been used in the course of our work, one a spectrofluorimeter modified with a flow cell and previously described[2], and the other a laboratory constructed model shown diagramatically in Fig. 1. This detector is designed with a right-angled optical configuration and uses filters to select the spectral regions employed for emission and excitation. The mountings for most of the components were machined from brass and are housed in a light-proof 30 × 30 × 23 cm steel box which contains the flow cell, the optics, the photomultiplier, the photomultiplier power supply, and the xenon lamp ignition circuit. Attached rigidly to the detector box is a second 15 × 30 × 13 cm steel box containing the xenon arc. It was found necessary to house the light source separately otherwise excessive heating of the photomultiplier would occur, leading to baseline drift.

The light source consists of an 150-W xenon arc lamp (Wotan) mounted by means of spring clips and PTFE bushes to a carriage providing vertical and horizontal adjustment of the arc position. Power is supplied via the mountings, the spring clips

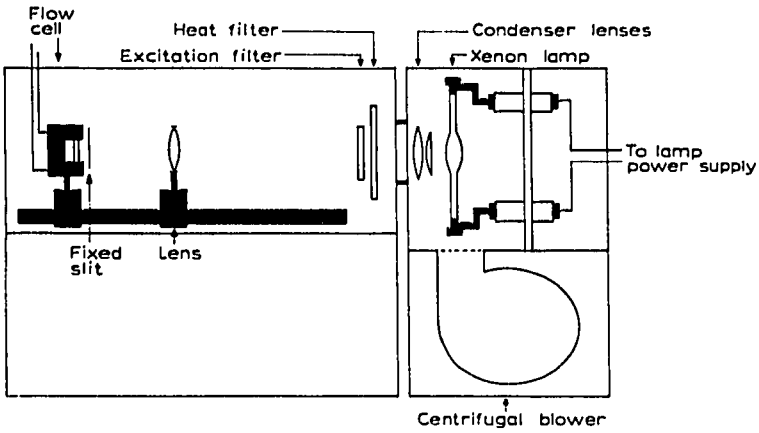

Fig. 1. Diagramatic sketch of the fluorimetric detector.

being supplemented by wired connections to the screw clamps provided on the lamp. The lamp is mounted vertically over the outlet of a 12-cm centrifugal blower, and the lamp housing is provided with light-baffled vents. Without cooling, the lamp showed marked instability. The lamp power supply is housed in a separate unit and supplies a regulated voltage at the rated lamp current (*i.e.*, 7.5 A).

Light from the lamp is passed through a two-element quartz condenser lens and is directed into the detector box. There the light passes, via a heat filter, through an appropriate excitation filter mounted in a sliding assembly which allows external changes of filter. The light is then focussed via a quartz lens on to the vertically mounted quartz tube of the flow cell.

The flow cell consists of a length of quartz tubing specially selected because of its low fluorescence (Supersil, available from Heraeus Quartz Fused Products Ltd., Shepperton, Great Britain, was found to be suitable). A 2.4-cm length of the tube, 3.5 mm O.D. and 1.5 mm I.D., cemented into a brass mount with an epoxy adhesive, is used in the detector. A 1/16-in. stainless-steel tubing of 0.25 mm I.D. was brazed into the cell mounting to act as solvent inlet and outlets. Only 12 mm of the quartz tube lies in the light path of the excitation beam, and those parts outside it were plugged with porous PTFE to minimise dead volume and to promote thorough mixing of the eluate as it enters the cell. Fixed slits of 1-mm width are placed in close proximity to the tube in both the excitation and emission light paths. It was found that these slits reduced light scatter from the surface of the tube, and enhanced the signal-to-noise ratio of the measured emission signal.

The emitted light passes through an interchangeable blocking filter housed in a slideable mount analogous to that of the excitation filter. This mount also acts as a shutter for the photomultiplier tube (RCA 1P28) which is placed immediately behind the filter. The photomultiplier output is fed to the recorder via a buffer amplifier which also provides zero suppression and a range of attenuations. Solid state circuitry of standard type was used in the design of the electrical components of the detector; details of these circuits are not included but will be given on request.

The optical filters used in this work have included a selection of interference filters covering the range 310–436 nm (available from Balzers High Vacuum Ltd., Berkhamp-

stead, Great Britain) and a variety of blocking filters with cut-offs up to *ca.* 460 nm. It is essential to use filters displaying high transmission in the selected spectral regions if the maximum sensitivity of fluorimetric detection is to be achieved.

Analysis

The chromatographic system described above was used to study both standard mixtures of polynuclear hydrocarbons and an extensive range of extracts from used engine oils. Oils were dissolved in methylene chloride (150 mg oil/ml of solvent) and 2-μl aliquots of the resulting solutions were injected on to the columns.

RESULTS AND DISCUSSION

In common with other reported work on small-particle-size silica gel[3] the columns packed with the microparticulate support display a very much higher efficiency than columns packed with comparable materials of larger particle size. Thus, whereas Corasil C_{18} (37–50 μ) gave plate heights of 1.6–0.8 mm for anthracene[2], the present columns give values of *ca.* 0.08 mm at an equivalent solvent velocity. This improved efficiency can lead to greatly enhanced resolution when complex mixtures are analysed. Fig. 2 shows, for example, the same oil solution examined on a column packed with Corasil C_{18} and a column packed with the material prepared in this laboratory. Although very much higher pressure drops are experienced with columns containing microparticulate packings, shorter columns can be used to achieve the same resolving power and this largely offsets any pressure disadvantages.

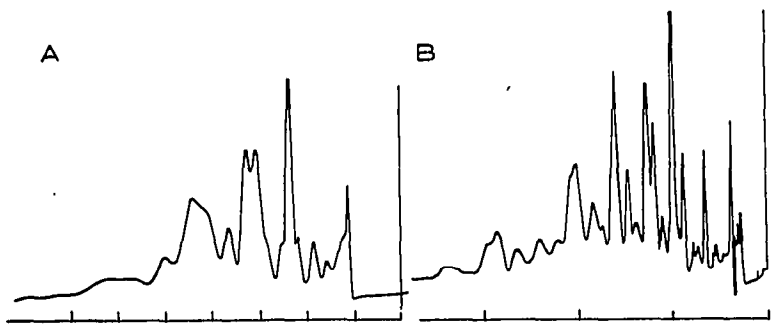

Fig. 2. Comparison of the chemically modified microparticulate silica with a commercial pellicular support of similar characteristics. (A) 6-ft. × ⅛-in.-O.D. column containing Corasil C_{18}. Solvent, methanol–water (3:1); flow-rate, 0.5 ml/min; pressure, 750 p.s.i. (B) 25-cm × ¼-in.-O.D. column containing a chemically modified silica (see text). Solvent, methanol–water (9:1); flow-rate, 1 ml/min; pressure, 1000 p.s.i. (The time intervals on the chromatograms are 5 min.)

The chemically bonded packing material gave a weight loss of 28% when heated to constant weight at 600°. If the preliminary hydrolysis of the silica was omitted or the reaction carried out at a lower reflux temperature, lower organic loadings resulted. This is a disadvantage because methanol–water mixtures of higher water content (and hence higher viscosity) are then necessary to achieve comparable separations of polynuclear hydrocarbons. Typical retention time data are shown in Table I. Determinations were carried out under isothermal conditions since they display

TABLE I

RELATIVE RETENTION TIME DATA FOR POLYNUCLEAR HYDROCARBONS

Column, 25 cm 7-μ silical gel modified with octadecyltrichlorosilane; solvent, methanol–water (9:1); flow-rate, 1 ml/min; temperature, 25°.

Compound	*Retention time relative to anthracene*
Naphthalene	51
Acridine	56
Acenaphthylene	56
Diphenyl	56
5,6-Benzoquinoline	66
Acenaphthene	72
Fluorene	76
Phenanthrene	84
Anthracene	100*
Fluoranthene	120
3,4-Benzacridine	126
Pyrene	135
1,2-Benzanthracene	230
Chrysene	277
Perylene	409
3,4-Benzofluoranthene	417
Benzo[*a*]pyrene	422
1,2,3,4-Dibenzanthracene	505
1,2,5,6-Dibenzanthracene	600
Benzo[*ghi*]perylene	875
1,2,3,4,-Dibenzopyrene	1280

* Retention time = 9.6 min.

temperature dependence. Fig. 3 shows separations of standard mixtures of polynuclear hydrocarbons using different solvent systems. There is a very marked difference in the apparent efficiency of the column depending on the solvent being used, but this effect has not been studied systematically. Choice of a solvent for a particular analysis depends on the nature of the components in the mixture. It is our practise to use methanol–water ratios of about 9:1 for the analysis of the polynuclear hydrocarbons present in engine oils, in which the materials of relatively low molecular weight predominate.

The preparative procedures described above have been used to prepare several columns and are sufficiently reproducible to be acceptable for routine analysis. Very little variation is encountered in relative retention time data between different columns but their chromatographic efficiencies do vary (*e.g.*, columns display number of theoretical plate values ranging between 3000–5000 based on the anthracene peak measured at a methanol–water (9:1) flow-rate of 1 ml/min). It is not clear whether these variations reflect differences in the packing or the injection. In our experience the injection procedure can make a marked difference to the resulting chromatogram. In all the work reported in this paper injections have been made on to some form of column insert, and the column was mounted vertically. These injection procedures give reproducible chromatograms but higher efficiencies were initially obtained from chromatograms derived from injections made directly into the packing material. This phenomenon was attri-

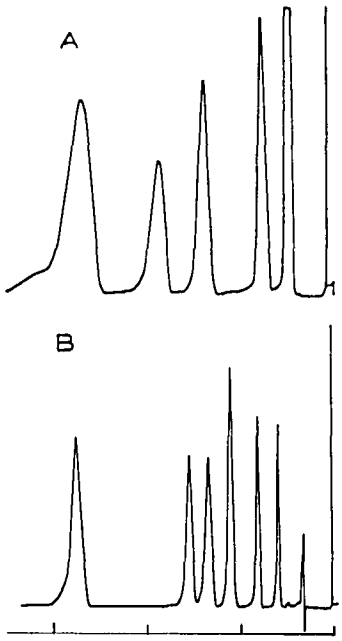

Fig. 3. Separation of polynuclear hydrocarbons on the chemically modified microparticulate silica. Column: 25 cm (see text). (A) Solvent, isooctane; flow-rate, 1 ml/min; pressure, 500 p.s.i. Compounds in order of elution: anthracene, 1,2-benzanthracene, benzo[*a*]pyrene, benzo[*ghi*]perylene, 1,2,3,4-dibenzopyrene. (B) Solvent, methanol–water (9:1); flow-rate, 1 ml/min; pressure, 1100 p.s.i. Compounds in order of elution: diphenyl, fluorene, anthracene, fluoranthene, pyrene, 1,2-benzanthracene.

buted to the "infinite diameter effect"[4] but did not offer an acceptable basis for routine operation as a conical void developed in the packing material after a few injections and this led to double peaking and peak asymmetry. Another disadvantage of on-column injection is that syringe needle blockage can readily occur with microparticulate packings. Oil and similar samples have a tendency to deposit carbon and other insoluble matter at the top of the column and column contamination can be avoided if a disposable insert is used. The use of $\frac{1}{4}$-in.-O.D. columns rather than the $\frac{1}{8}$-in.-O.D. used previously[2] was adopted in an attempt to utilise the "infinite diameter effect". In practise it seems doubtful if this effect is operative when injection procedures such as those described above are used. Nevertheless, there does not appear to be any disadvantage to the use of wider-bore columns.

The variation in plate height as a function of solvent velocity is shown in Fig. 4 for three compounds of different k' values. These curves indicate that there is an appreciable fall off of column efficiency as the solvent velocity increases, and it is our experience with this type of column that a change in solvent composition rather than an increase in solvent flow-rate is the most effective way to effect a more rapid analysis.

Fluorimetry provides a convenient method of improving both the selectivity and sensitivity of liquid chromatographic analyses of polynuclear hydrocarbon mixtures. Fig. 5 illustrates the potential for selectivity. The six chromatograms shown were each obtained by carrying out identical separations of a six-component mixture

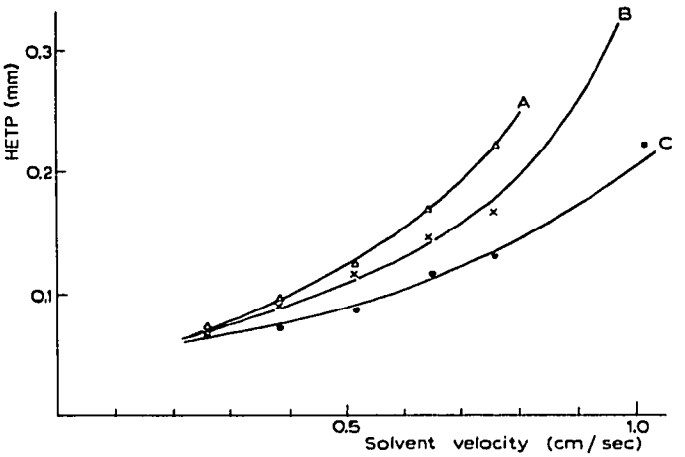

Fig. 4. Column characteristics for a chemically modified microparticulate silica. Column, 25 cm (see text); solvent, methanol–water (9:1). A = Benzo[*a*]pyrene, $k' = 19$; B = 1,2-benzanthracene, $k' = 6.5$; C = anthracene, $k' = 2$.

on the column system described. In each case the eluent was monitored by being passed through the flow cell in the modified MPF 2A spectrofluorimeter, which was operated under different excitation and emission conditions. The six compounds in the mixture each have rather different fluorescence characteristics (see Table II).

The appreciable variations encountered in the appearance of the chromatograms can be readily explained on the basis of the differences in the fluorescence properties of the different compounds. The combination of selective fluorimetric detection and characteristic retention time data appears to offer a powerful method for qualitatively identifying components in a mixture of polynuclear hydrocarbons. In our own work identification of individual compounds is not important because the objective of the work is to use the chromatogram as a "fingerprint". Nevertheless, with the complex mixtures encountered in engine oils we find that monitoring under different fluorescence conditions yields chromatograms that vary widely and this greatly simplifies comparison of samples. In Fig. 6, for example, the same oil has been chromatographed under identical conditions but the eluate has been monitored by a UV absorption detector and a fluorimetric detector operated under two different sets of conditions.

TABLE II

FLUORESCENCE CHARACTERISTICS OF THE SIX-COMPONENT MIXTURE OF FIG. 5

Compound	*Excitation maximum (nm)*	*Emission maximum (nm)*
Naphthalene	275	320, 330
Biphenyl	250	305, 315
Anthracene	340, 357, 375	380, 400, 420, 448
Fluoranthene	410	402, 415, 441, 468
Pyrene	320, 337	370, 385, 395
Perylene	380, 400, 438	445, 465, 500

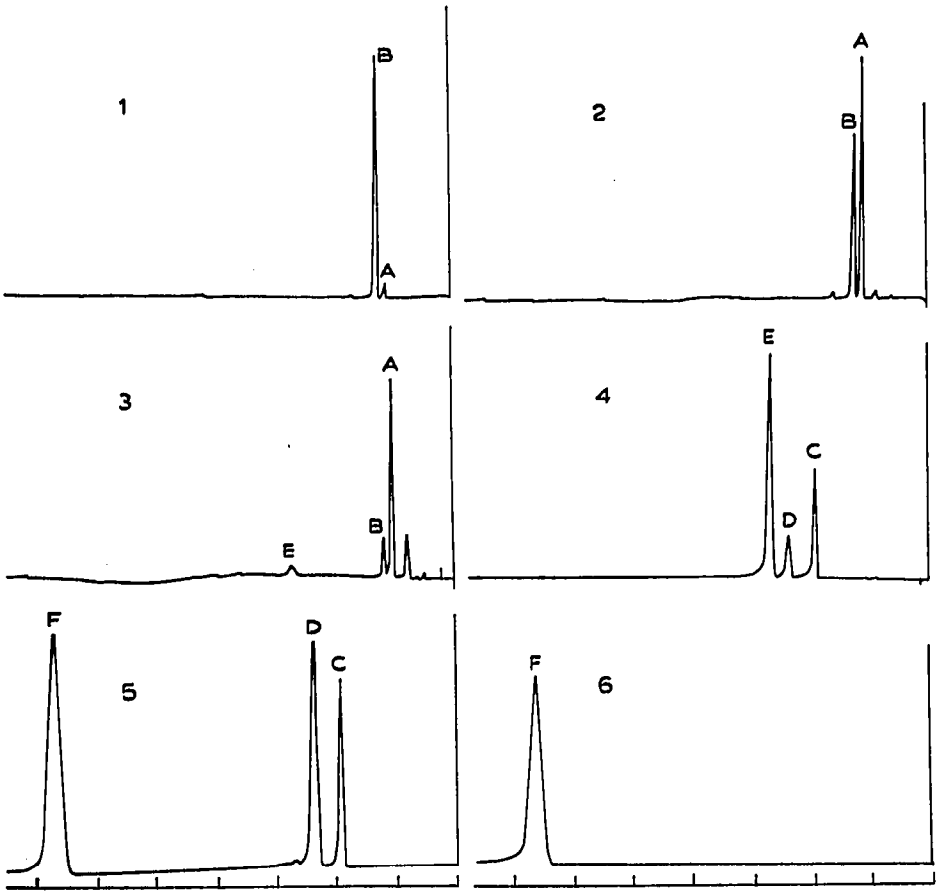

Fig. 5. Illustration of the selectivity of fluorimetric detection. Column, (25 cm × ¼-in.-O.D.) containing a chemically modified microparticulate silica. Solvent, methanol–water (9:1). A = Naphthalene (42 ng); B = diphenyl (42 ng); C = anthracene (26 ng); D = fluoranthene (20 ng); E = pyrene (48 ng); F = perylene (50 ng).

EXCITATION CONDITIONS

Chromatogram	*Excitation wavelength (nm)*	*Emission wavelength (nm)*
1	245	305
2	275	320
3	280	340
4	335	385
5	350	430
6	410	470

No systematic work has been carried out to assess the sensitivity of the fluorimetric detectors because each compound would be required to be run under optimum excitation and emission conditions for the results to be meaningful. In general 10–100 pg of each compound can be detected after elution, and the response is linear over a wide range. The laboratory-built detector provides a relatively inexpensive

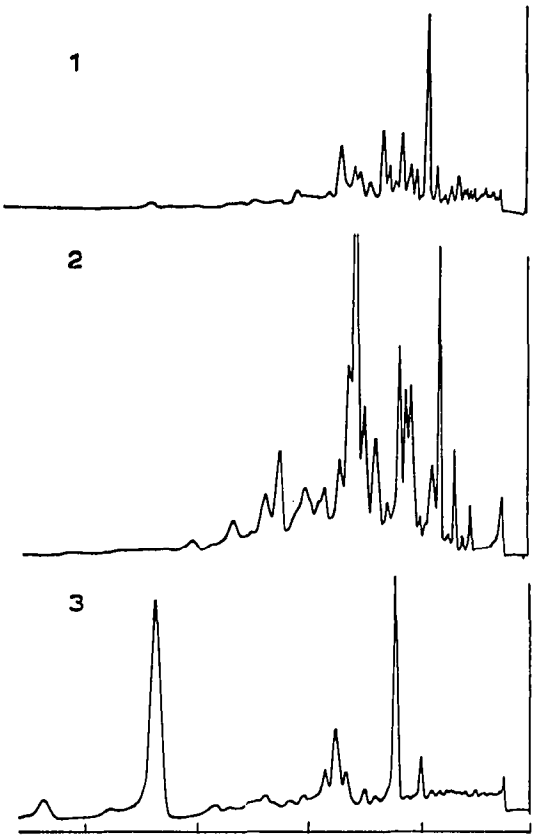

Fig. 6. Use of UV and fluorimetric detection for monitoring engine oil chromatograms. Column, 25 cm × ¼-in.-O.D., containing a chemically modified microparticulate silica. Solvent, methanol–water (9:1). The time intervals on the bottom scale are 5 min.

DETECTION CONDITIONS

Chromatogram	*Detection conditions*
1	UV at 254 nm
2	fluorimetric $\lambda_{ex.} = 275$ nm, $\lambda_{em.} = 320$ nm
3	fluorimetric $\lambda_{ex.} = 360$ nm, $\lambda_{em.} = 460$ nm

alternative to the modified commercial spectrofluorimeter. Its performance is comparable with the latter when operated under similar conditions, but with the limited range of filters we currently have available it has not been possible to carry out an assessment over the complete wavelength range.

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