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POLYCYCLIC AROMATIC HYDROCARBONS IN THE ENVIRONMENT: HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING CHEMICALLY MODIFIED COLUMNS*

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SUMMARY

A simple and rapid procedure for the separation and determination of a number of polycyclic aromatic hydrocarbons (PAHs) in environmental dust samples is described. An ultrasonic extraction method was used to separate the organic compounds from the dust particles. Only small amounts of dust and solvents are needed and the extraction requires only a few minutes. Compounds that would interfere in UV detection after high-performance liquid chromatography (HPLC) are separated from the aromatic fraction by preparative thin-layer chromatography on silica gel. The chromatographic behaviour of the carcinogenic hydrocarbons on several stationary phases in HPLC was investigated. A comparison of the chromatographic data showed that silica gel chemically modified with NO_2 groups gives the best selectivity. Nanogram amounts of PAHs were easily detected by using a UV monitor.

INTRODUCTION

Because of their carcinogenic activity, polycyclic aromatic hydrocarbons (PAHs) belong to the most intensely studied compounds in environmental samples that come into contact with the human organism. These aromatic hydrocarbons are generated by incomplete combustion processes, which occur wherever wood, coal or oil is burned and can therefore be found in very complex mixtures almost everywhere in the environment. A number of different analytical methods have been developed, all of which involve a combination of techniques such as solvent extraction, pre-cleaning steps such as liquid–liquid partition and column and thin-layer chromatography (TLC) and, for the final determination, various types of spectroscopy and chromatography. Many of these operations are time consuming and require considerable analytical experience in carrying out the individual steps.

To match the practical requirements of environmental chemistry, a suitable analytical procedure for the separation of PAHs should be simple, rapid and selective. A fundamental problem is the recovery of the organic fraction that adheres to the dust particles. The most common procedure for this is Soxhlet extraction, but this

* This paper is dedicated to Prof. Dr. W. Limontschew on the occasion of his 60th birthday.

method has the disadvantages that large amounts of samples and solvent are needed and the extraction period is long. These drawbacks can be avoided by using ultrasonic extraction.

As already mentioned, there are several techniques for the separation of the PAHs from the very complex mixtures of the organic fraction after pyrolysis. One of the simplest methods is preparative TLC. By applying this method, reproducibly pure fractions of the aromatics can be obtained that can be used immediately in HPLC analysis and the subsequent UV detection. Many workers have already described the use of HPLC for the determination of carcinogenic PAHs[1–15], mostly using reversed-phase chromatography on C-8 or C-18 supports. In numerous instances the selectivity of the separation of PAHs in environmental samples was not satisfactory, as some of the possible isomers showed poor resolution. For this reason, some column packings have been tested and the chromatographic behaviour of the aromatics has been investigated. A combination of microparticulate columns with high performance and selective UV detection has made it possible to develop a reliable procedure for the determination of some aromatic species.

EXPERIMENTAL

The PAH standards used were obtained from Fluka (Buchs, Switzerland), EGA-Chemie (Steinheim, G.F.R.) and private sources. For TLC, commercially available 20 × 20 cm Si 60 plates with 0.25 mm thick layers from Merck (Darmstadt, G.F.R.) were used. All other chemicals used were of analytical-reagent grade and, if not stated otherwise, were obtained from Merck.

To check the single extraction steps, radioactively labelled [7,10-^{14}C]benzo[*a*]pyrene from the Radiochemical Centre (Amersham, Great Britain), was used. The measurement of the activities was carried out with a Beckman β Mate II liquid scintillation counter. The required scintillation cocktails contained 10 g/l of butyl-PBD (Beckman Instruments, Fullerton, Calif., U.S.A.) in toluene and the activities were calculated by the external standard ratio method.

The column supports used for HPLC were LiChrosorb RP-18 (5 μm), LiChrosorb NH_2 (10 μm) (both from Merck) and Nucleosil 5 NO_2 (from Macherey, Nagel & Co., Düren, G.F.R.). The columns were wet packed according to the slurry method. A pre-tested column (SS 200/6/4/Nucleosil 5 NO_2), generously supplied by Macherey, Nagel & Co., produced the same results as the self-packed columns.

The HPLC system consisted of a Waters M 6000 pump, a variable-volume injection system (Waters U6K) and an SF 770 variable-wavelength UV detector from Schoeffel Instrument Corp. For non-selective measurements, *i.e.*, establishing the elution characteristics of selected PAH, a wavelength of 296 nm was chosen, because a number of these compounds show strong absorption in this region. For the detection of some selected PAHs in dust samples, a wavelength of 384 nm was used so as to be selective for both the separation and the detection.

RESULTS AND DISCUSSION

Extraction procedure

Dust samples from the ventilation system of a tunnel (Katschberg tunnel,

Tauernautobahn, Salzburg, Austria) that had been exposed to automobile exhaust gases were taken as typical specimens of PAH-containing material. In order to study the extraction of these compounds ^{14}C-labelled benzo[*a*]pyrene (B*a*P) was used. Similar behaviour of the other PAHs was assumed because of their chemical similarity. The lack of relevant standard materials makes it impossible to achieve absolute results, and therefore the extraction efficiency was determined as follows.

A certain amount of cyclohexane containing [^{14}C]B*a*P was added to the dry dust samples in a test-tube. The volume of cyclohexane solution must be sufficient to wet all of the material and to penetrate the pores of the particles. The samples were then kept at 50° until they were completely dry again. By means of this procedure, the total surface of the dust was homogeneously covered with [^{14}C]B*a*P. For each Soxhlet extraction, 1 g of this type of prepared dust was weighed out.

Fig. 1 shows the extraction curve for 10 μg of [^{14}C]B*a*P per gram of dust as a function of the time of extraction. Benzene (100 ml) was used as the solvent.

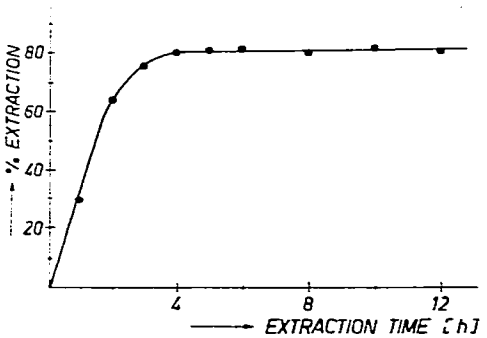

Fig. 1. Extraction curve for [^{14}C]B*a*P prepared dust in a Soxhlet apparatus.

The main advantage of this procedure is the relatively high yield (81 %) after about 6 h of extraction. A longer time of extraction did not increase the efficiency noticeably. The disadvantage of this method is the length of time required and the need for large amounts of sample and solvent. In addition, extensive purification of the solvents is necessary as impurities interfere in the subsequent analysis.

An ultrasonic extraction step has been devised that permits the PAH, to be extracted with smaller amounts of sample and solvent in a shorter time. The best results were obtained when 200 mg dust were placed in vials with screw-caps and 1 ml of benzene was added to each. This mixture was placed in an ultrasonic bath for 5 min. After centrifugation, 800 μl of the solvent can be withdrawn and used for scintillation measurement or further analysis. Table I shows the results of the repeated extraction of 10 μg of B*a*P from a sample of 200 mg of dust using 1 ml of benzene per extraction step.

The yield is not increased if the period of ultrasonic treatment is extended. After three extraction steps of 5 min each, the same recovery as that obtained with the Soxhlet apparatus is achieved. These recoveries are reproducible and, in the range between 50 ng and 100 μg of B*a*P per 200 mg of dust they do not depend on the amount of PAHs.

TABLE I

RECOVERY OF 10 μg OF B*a*P FROM DUST PARTICLES AFTER REPEATED ULTRASONIC EXTRACTION

Fraction	*BaP found (μg)*	*Total recovery (%)*
1st ml	4.65 ± 0.19	46.5
2nd ml	2.39 ± 0.10	70.4
3rd ml	1.27 ± 0.06	83.1
4th ml	0.79 ± 0.04	91.0

Clean-up procedure

Because of the presence of numerous nitrogen-, sulphur- and oxygen-containing substances that are generated by incomplete combustion and would interfere in UV or fluorescence detection after HPLC separation, the dust extracts have to be pre-cleaned. There are various possibilities for obtaining pure fractions of the aromatic hydrocarbons. A pre-cleaning procedure using chromatographic columns involves a lengthy equilibration and includes an undesirable dilution caused by the relatively large elution volume.

In this work, a TLC separation followed by extraction of the PAHs from the plates was chosen, and the plates were discarded after use[16]. The advantage of this technique is the small volume of the extracts from the plates, which obviates the need for concentration. For this purpose, a 5 cm line of 800 μl of an extract is applied on to silica gel Si 60 plates together with a test mixture containing a number of PAHs and developed with cyclohexane–benzene (1:1.5). As shown in Fig. 2, all PAHs of interest can be found in a small region with R_F values between 0.65 and 0.75. Less polar compounds, *i.e.*, aliphatics, are found near the front whereas more polar substances such as heterocyclics and other nitrogen-, oxygen- and sulphur-containing compounds with low R_F values are detected near the start.

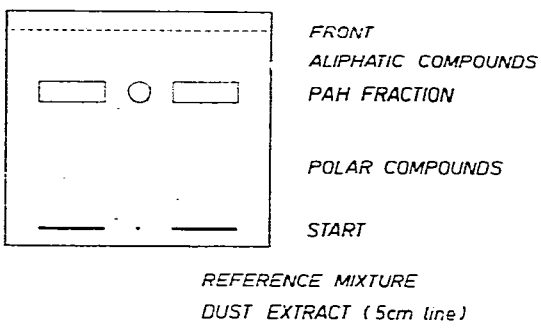

Fig. 2. TLC of organic dust extracts on silica gel. Layer, 0.25 mm; developing solvent, cyclohexane–benzene (1:1.5). Reference mixture contains benzo[*a*]pyrene, perylene, benzo[*k*]fluoranthene, dibenzo[*a,h*]anthracene, coronene, indeno[1,2,3-*cd*]pyrene and benzo[*a*]anthracene.

The zone with the aromatic fraction is marked under UV light at 254 nm and scraped from the plate into a small test-tube. Extraction is again carried out in an ultrasonic bath for 5 min. [^{14}C]B*a*P was used to establish optimal conditions for this step. Two different extracts, one with cyclohexane as solvent for non-polar mobile phases and the other with methanol for water-containing mobile phases, were prepared for the reported HPLC separations. The recoveries of B*a*P are shown in Table I.

TABLE II

ULTRASONIC EXTRACTION OF 8.80 μg OF BaP FROM SILICA GEL AFTER TLC

Fraction	*Cyclohexane*		*Methanol*	
	BaP found (μg)	*Total recovery (%)*	*BaP found (μg)*	*Total recovery (%)*
1st ml	3.90	44.3	5.05	57.4
2nd ml	2.20	69.3	2.15	81.8
3rd ml	1.10	81.8	0.85	91.5
4th ml	0.60	88.6	0.35	95.5

It is interesting that a close analogy exists between the extraction of BaP from dust and from silica gel. This indicates that that both materials might have similar chemical surface and structural properties.

The yield increases with increasing polarity of the solvent, but in extraction the amount of interfering polar compounds also increases. For subsequent analysis by means of HPLC, the solvent has to be readily miscible with the mobile phase, must not interfere in detection and, above all, should dissolve the PAH. These requirements are best met by cyclohexane as a non-polar and methanol as a polar solvent. After extraction, the samples are centrifuged at 2100 *g* for 5 min and an aliquot of the clear, supernatant liquid can be injected directly into the HPLC system.

HPLC separations

A serious problem when trying to separate the carcinogenic PAHs that actually occur in environmental samples is the chemical selectivity of liquid chromatography. The most common separation system is reversed-phase chromatography, but it is not selective enough to solve this particular problem. Therefore, the separation characteristics of chemically modified supports such as LiChrosorb NH_2 and Nucleosil NO_2 were examined. Both were tested with mixtures of solvents covering a wide range of polarity. The best results were obtained with non-polar solvents. The choice of the mobile phase gave no problems as a distribution mechanism is the main factor for the separation of the PAHs on these modified supports. The polarity of the mobile phase is more decisive than the type of functional groups present, and retention characteristics remain virtually constant irrespective of the composition of a mixture of non-polar and slightly polar liquids.

In Table III, the k' values of various PAHs on both columns are compared with those on LiChrosorb RP-18. Mobile phases that gave good resolution and rapid analyses were selected. The most suitable solvents are isooctane dried over alumina for use with LiChrosorb NH_2 and isooctane containing 10% of dichloromethane for use with Nucleosil NO_2. For the analysis of PAHs on LiChrosorb RP-18, acetonitrile–water (85:15) was chosen. The separation increased with an increase in the water content, but this was offset by a rapid increase in retention volume. In no instance was a temperature higher than ambient necessary for chromatography.

Depending on the wavelength, selected PAHs can be seen on UV detection as they generally have well defined adsorption maxima with narrow bandwidths[17]. For analysis of dust extracts a wavelength of 384 nm was chosen. Here the PAHs that are generally considered to be less carcinogenic show no or little UV response, whereas a

TABLE III

k' VALUES OF SEVERAL PAHs ON DIFFERENT COLUMNS

LiChrosorb RP-18 (5 μm), 150 × 3.2 mm, mobile phase acetonitrile–water (85:15); Nucleosil 5 NO_2, 150 × 3.2 mm, mobile phase isooctane–dichloromethane (9:1); LiChrosorb NH_2 (10 μm), 250 × 3.2 mm, mobile phase isooctane dried over alumina.

Compound	k'		
	LiChrosorb RP-18	*Nucleosil NO_2*	*LiChrosorb NH_2*
Fluoranthene	2.09	1.45	1.48
Pyrene	2.71	1.47	1.18
Triphenylene	2.82	2.33	1.86
11H-Benzo[*a*]fluorene	2.88	1.25	1.48
Benzo[*a*]anthracene	3.29	1.94	2.12
Chrysene	3.35	2.16	2.00
Benzo[*b*]fluoranthene	4.89	3.05	2.72
Perylene	4.99	3.37	2.07
Benzo[*e*]pyrene	5.05	3.19	2.72
Benzo[*k*]fluoranthene	5.33	2.44	2.78
Dibenzo[*a,c*]anthracene	5.84	4.52	3.55
Benzo[*a*]pyrene	6.17	2.79	2.57
Dibenzo[*a,h*]anthracene	7.01	3.64	3.67
3-Methylcholanthrene	9.21	2.09	2.32
Indeno[1,2,3-*cd*]pyrene	9.29	3.87	3.38
Benzo[*ghi*]perylene	9.55	4.32	3.52
Coronene	18.96	6.45	4.60

number of pentacyclic aromatics still have absorption coefficients that give detection limits in the nanogram range. Accordingly, the resolution between B*a*P, benzo[*k*]fluoranthene, 3-methylcholanthrene, indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene has to be as good as possible. Coronene, which always appears last, can be seen by altering the wavelength to 333 nm at the end of the chromatogram. When comparing the k' values of these compounds, one can see that Nucleosil NO_2 has the best separation characteristics under the conditions described. Chromatography on LiChrosorb RP-18 gives a poor resolution of 3-methylcholanthrene, benzo[*ghi*]perylene and indeno [1,2,3-*cd*]pyrene; on Nucleosil NO_2, however, they are well separated. In both instances B*a*P and benzo[*k*]fluoranthene can be defined separately. Further, the possibility of the separation of the isomeric benzopyrenes is interesting. LiChrosorb NH_2 gives a poor resolution of B*a*P and benzo[*k*]fluoranthene and indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene appear as one peak.

Figs. 3 and 4 show the chromatograms obtained on LiChrosorb RP-18 and Nucleosil NO_2, respectively.

For the quantitative determination of these compounds in the tunnel dust, a Nucleosil NO_2 column was used. As the extraction and pre-cleaning procedures were developed only with the help of [^{14}C]B*a*P, all other PAHs of interest were added to the dust as they could possibly show slightly deviating results. This procedure was carried out in the same way as that used for the extraction of [^{14}C]B*a*P. Both the original and the pre-treated samples were subjected to repeated analyses and hus chromatograms with different peak heights and constant retention parameters were obtained. The difference in the peak heights was considered to be equivalent to the

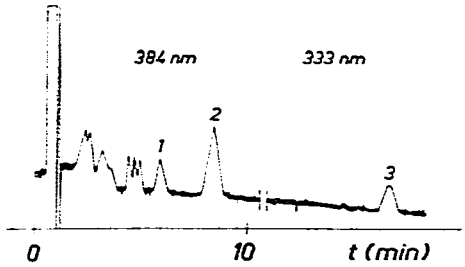

Fig. 3. Chromatography of the aromatic fraction of the dust extract on LiChrosorb RP-18, (5 μm), 150 × 3.2 mm. Mobile phase: acetonitrile–water (85:15), flow-rate 1 ml/min. Peaks: 1 = benzo[*a*]-pyrene; 2 = dibenzo[*a*,*h*]anthracene, 3-methylcholanthrene, indeno[1,2,3-*cd*]pyrene; 3 = coronene.

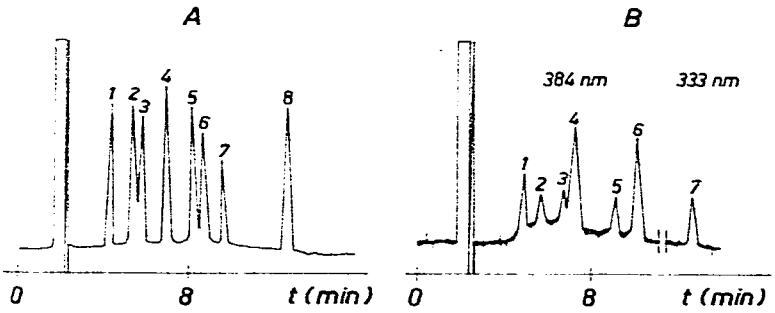

Fig. 4. Separation of PAH on a Nucleosil 5 NO_2 column, 150 × 3.2 mm. Mobile phase: isooctane–dichloromethane (9:1), flow-rate 1 ml/min. (A) Chromatogram of a mixture of PAH standards. Peaks: 1 = pyrene; 2 = benzo[*a*]anthracene; 3 = 3-methylcholanthrene; 4 = benzo[*a*]pyrene; 5 = dibenzo[*a*,*h*]anthracene; 6 = indeno[1,2,3-*cd*]pyrene; 7 = benzo[*ghi*]perylene; 8 = coronene. (B) Chromatography of a pre-cleaned dust extract. Peaks: 1 = unknown; 2 = 3-methylcholanthrene; 3 = benzo[*k*]fluoranthene; 4 = benzo[*a*]pyrene; 5 = indeno[1,2,3-*cd*]pyrene; 6 = benzo[*ghi*]-perylene; 7 = coronene.

amount of PAH added to the dust. Each analysis was repeated seven times. The results together with the detection limits are shown in Table IV.

A reason for the low content of PAHs found in the analysed tunnel dust is that at the time of sampling the tunnel had only recently been opened and was poorly

TABLE IV

DETERMINATION OF SEVERAL PAHs IN TUNNEL DUST USING A NUCLEOSIL 5 NO_2 COLUMN

Reported contents and detection limits were evaluated at 384 nm except those for coronene (333 nm).

Compound	*Detection limit (ng)*	*Content in dust (ng/g)*	*Relative standard deviation (%)*
Benzo[*a*]pyrene	0.44	370	9.3
Benzo[*k*]fluoranthene	1.87	550	13.7
3-Methylcholanthrene	3.23	260	17.5
Indeno[1,2,3-*cd*]pyrene	1.34	360	10.8
Benzo[*ghi*]perylene	0.96	450	9.5
Coronene	0.99	500	9.7

frequented. For the calculation of the detection limits a peak height that corresponds to twice the baseline noise was taken.

The selectivity and sensitivity obtained with a UV detector could be improved by using a suitable fluorescence detector.

CONCLUSION

The results show that liquid chromatography is a reliable method for solving difficult separation problems because of its selectivity. In many instances, classical absorption chromatography on Silica gel or alumina or reversed-phase chromatography can be supplemented or replaced with a system with chemically modified column supports. Thus, by selecting suitable stationary and mobile phases novel possibilities for the separation of chemically very similar PAHs can be developed. The application of such a chromatographic system has been demonstrated by the analysis of some potential carcinogenic PAHs in complex mixtures and environmental samples.

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