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PRESEPARATION OF POLAR, SUBSTITUTED, POLYCYCLIC AROMATIC HYDROCARBONS IN AEROSOL EXTRACTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON ACIDIC- OR BASIC-BUFFERED SILICA

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SUMMARY

Complex air sample extracts were prefractionated on acidic- and basic-buffered silica. Compound overlap between acidic and basic polar substances (*e.g.* substituted polycyclic aromatic hydrocarbons) was greatly reduced, facilitating identification by gas chromatography–mass spectrometry. Furthermore, additional information on compound acidity and polarity is obtained, which helps to characterize compounds with identical electron-impact mass spectra but different structures.

INTRODUCTION

Prefractionation and clean-up techniques are an important part of many analytical methods that determine single compounds or compound groups in complex sample matrices. High-performance liquid chromatography (HPLC) is frequently used to preseparate such samples into fractions of different polarity. This additional information on compound polarity is of great value when unknown substances have to be identified by, for example, gas chromatography–mass spectrometry (GC–MS).

Recently, a method has been published by Schwarzenbach, who proposed HPLC on acidic- or basic-buffered silica for the separation of polar compounds[1]. He showed that losses of such compounds by irreversible adsorption are greatly reduced on such surfaces.

Complex air sample extracts from industrial sites and indoor air samples often contain a large variety of both acidic and basic polar compounds. Groups of substances with different acidity are present in the most polar fractions. They are eluted in the same fraction from normal silica and frequently interfere with identification by GC–MS due to compound overlap. It was therefore of interest to investigate whether preseparation on buffered silica is able to increase the number of identifiable substances. Furthermore, a large variety of substituted polycyclic aromatic hydrocarbons (PAHs) with different acidities are present in many air sample extracts. Substituted PAHs with the same elemental composition often give identical electron-impact (EI) mass spectra. Further information on the polarity and acidity of the compound is therefore a great help for proper identification. Different examples are given, which show that preseparation on buffered silica is able to supply this infor-

mation. In addition, losses of polar, substituted PAHs are greatly reduced by this technique, and more information on sample composition is obtained, even from very complex air sample extracts.

EXPERIMENTAL

The same approach as described by Stray et al.[2] has been used for air sampling, extraction, and compound identification by GC–MS:

(1) High-volume sampling with glass-fibre filters of 142 mm diameter (Gelman type 61635).

(2) Soxhlet extraction with liquid carbon dioxide[2].

(3) Derivatization with diazomethane, if necessary.

(4) Prefractionation by HPLC of half of the extract (redissolved in 300 μl of dichloromethane) on basic- and acidic-buffered silica, respectively.

Five acidic fractions and five basic fractions; 250 × 4.6 mm I.D. column, LiChrosorb Si 60, 5 μm (Merck, Darmstadt, F.R.G.); 0.1 M Na$_2$HPO$_4$ (pH 8.0) or 0.1 M NaHSO$_4$ (pH 1.0). Gradient from hexane to 60% dichloromethane for 20 min, then 100% dichloromethane. Flow-rate, 2 ml/min.

(5) High-resolution gas chromatography on OV-1 (fused-silica capillary column, 30 m × 0.3 mm I.D., 0.15 μm film thickness).

(6) Identification by electron-impact mass spectrometry at 70 eV or CH$_4$-NICI mass spectrometry (0.4 Torr; source temperature, 200°C; electron energy 95 eV on a Hewlett-Packard 5985/87A mass spectrometer)[3].

HPLC columns of 250 × 4.6 mm I.D. were packed with acidic- or basic-buffered silica, following the procedure of Schwarzenbach[1]. LiChrosorb Si 60 (Merck, 5 μm particle diameter) was used as the starting material. A 0.1 M solution of Na$_2$HPO$_4$ (pH 8.0) was employed for the basic coating and 0.1 M NaHSO$_4$ (pH 1.0) for the acidic layer. Silica (5 g) was added to ca. 50 ml of the solution to form a fluid slurry. The buffer solution was forced into the pores by using an ultrasonic bath and vacuum for about 2 min.

After filtration, the wet material was dried at 80°C under vacuum, overnight. A hexane slurry was used to pack the columns. Occasionally, it was not possible to

TABLE I

RECOVERY OF SOME SUBSTITUTED AND HETEROCYCLIC PAHs ON ACIDIC- AND BASIC-BUFFERED SILICA

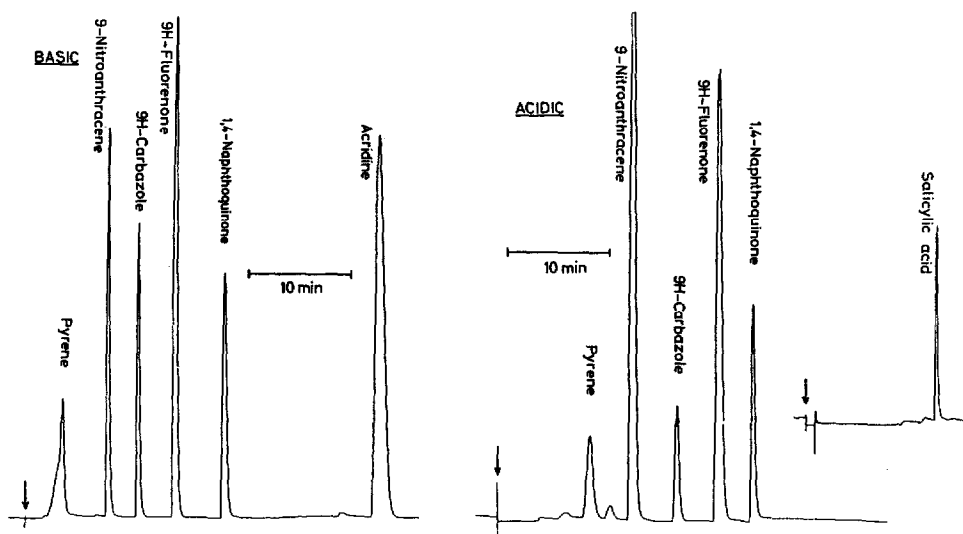| Compound	Acidic buffered			Basic buffered		
	n	$\bar{x}$ (%)	S_r (%)	n	$\bar{x}$ (%)	S_r (%)
1,4-Naphthoquinone	3	82.3	8.6	3	83.7	3.0
9H-Fluorenone	3	0.8	1.1	3	93.1	3.1
Acridine	Not eluted			3	94.1	4.3
9H-Carbazole	3	91.5	5.2	3	97.9	6.6
Pyrene	3	89.8	4.3	3	92.1	2.6
9-Nitroanthracene	3	81.3	3.2	3	87.2	4.1
Salicylic acid	3	106.7	15.9	Not eluted		

Fig. 1. Elution sequence of the test mixture from basic- and acidic-buffered silica. Acridine is not eluted from the acidic-buffered column. Concentration: 100–300 ng compound/μl; volume injected: 100 μl.

form a suitable slurry with hexane. Other apolar solvents are probably more suitable for column packing but were not tried. In general, no problem was encountered in the preparation of acidic-buffered silica columns, but only half of the columns packed with basic-buffered silica really had a basic coating. The relatively high pH value of the coating, which is at the upper limit for silica, might be an explanation of the problem. A mixture of 1,4-naphthoquinone, 9H-fluorenone, acridine (a basic aza-arene), 9H-carbazole, pyrene, and 9-nitroanthracene in dichloromethane was used to test the column performance (concentration ca. 100–300 ng/μl). The shape of the peak for salicylic acid was an additional test criterion for the acidic column (100 ng/μl in dichloromethane). About 100–200 μl were injected and separated with the solvent gradient given in Table I.

    The following fractions were taken: fraction 1 (parent and methylated PAH), fraction 2 (nitrated PAHs), fraction 3 (carbazoles), fraction 4 (keto-PAHs, hydroxy-PAHs, aza-arenes, fatty acid esters, phthalates etc.), fraction 5 (fatty acids, aza-arenes, etc.).

RESULTS AND DISCUSSION

    As can be seen from Fig. 1, the test compounds were eluted in the same order as from normal, activated silica[2]. The separation of carbazoles from keto- and ni-tro-PAHs is highly dependent on the degree of activation of the silica surface and on the water content of the solvent. With properly dried solvents, the separation behaviour of both acidic- and basic-buffered silica remained stable for months when the solvent gradient described above was used. 9-Nitroanthracene is a rather labile compound and is easily oxidized to 9H-anthracenone on highly active surfaces. No such effects could be observed with either column type. The recovery rates for the test compounds were in the order of 85–95% (Table I).
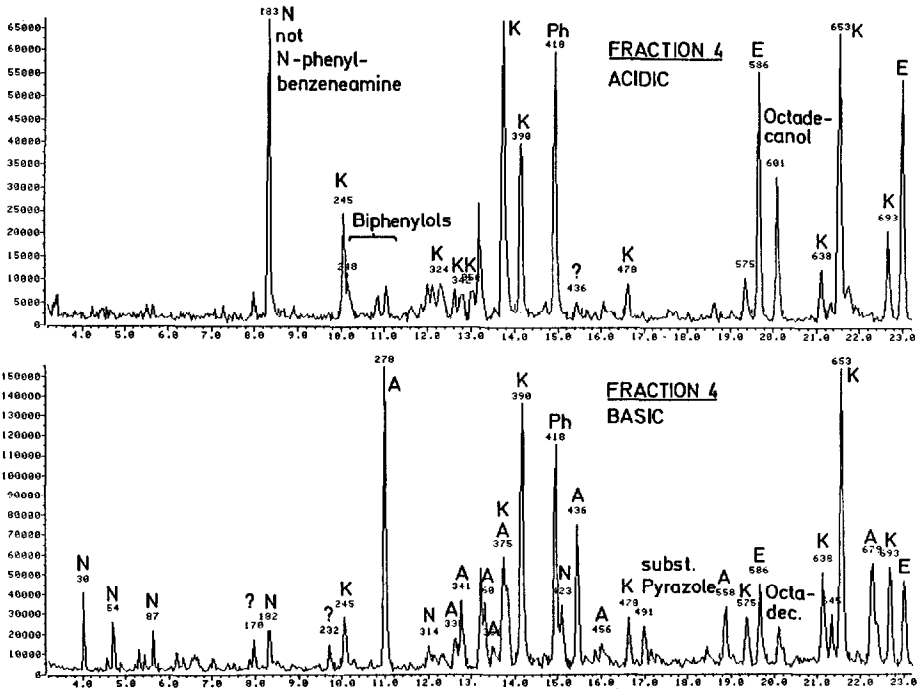
Fig. 2. Comparison of the total ion current chromatograms of an indoor air sample extract from an aluminium smelter, prefractionated on basic- and acidic-buffered silica. N = nitrogen-containing compounds, A = aza-arenes, K = keto-PAHs, Ph = phthalates, E = fatty acid esters. Temperature programme: 40°C, 2 min; 40–130°C, 30°C/min; 130–280°C, 5°C/min.
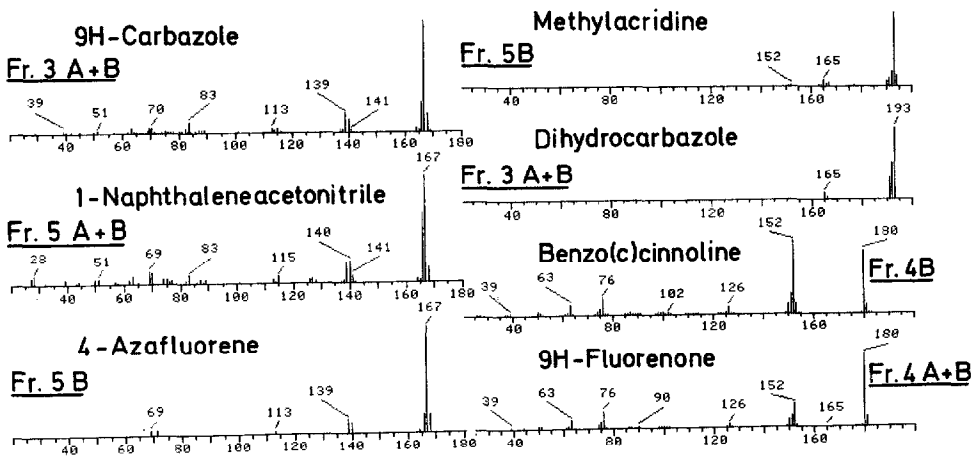

Fig. 3. Mass spectra of some substituted PAHs that cannot be identified without additional information on polarity and acidity. The HPLC fractions in which the compounds were found, are indicated.
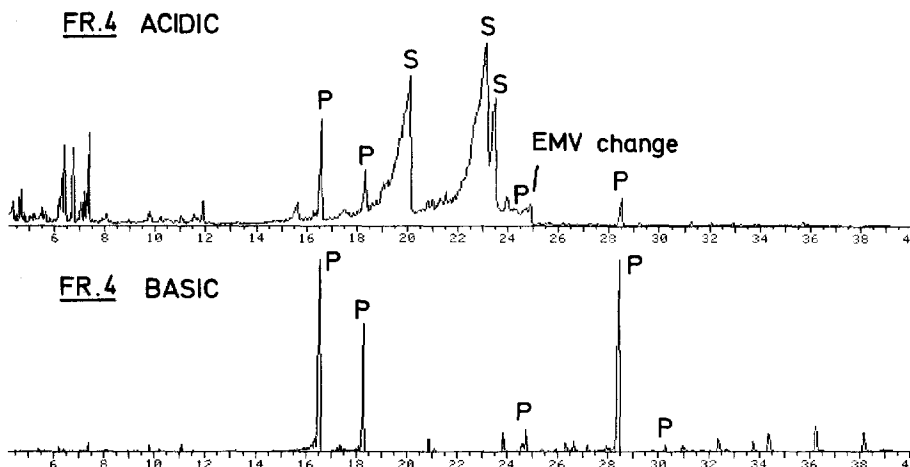
Fig. 4. Total ion current chromatogram of fraction 4 of an indoor air sample extract. Phthalates can easily be quantified in the basic fraction without interference from fatty acids. S = fatty acids, P = phthalates.

Furthermore, the retention of basic compounds is greatly reduced on the basic silica support. The same is valid for acidic compounds separated on acidic-buffered silica. Therefore, all substances could be eluted with solvents of moderate solvent strengths (e.g. dichloromethane). This considerably reduced the risk of washing out the salt layer. The advantage of preseparating complex air sample extracts on buffered silica is demonstrated by the following examples.

An indoor air sample extract from an aluminium smelter contained a large variety of neutral, acid and basic compounds. In Fig. 2, the medium polar fractions eluted from both acidic- and basic-buffered silica are compared. Neutral compounds, such as keto-PAHs and phthalates, were present in both fractions. The eluate from the acidic column contained hydroxy-PAHs and larger amounts of fatty acid esters and alcohols, while the basic fraction was dominated by aza-arenes, pyrazoles and pyridines. Overlap of compounds was reduced considerably, compared with fractionation on non-treated silica, and this faciliated the identification of the compounds.

Furthermore, additional information was obtained on the polarity and acidic/basic properties of each compound. This helped to identify those structural isomers with virtually identical EI mass spectra. Some examples are given in Fig. 3. 9H-Carbazole was eluted in fraction 3 (acidic and basic), while 1-naphthaleneacetonitrile was found in fraction 5 (acidic + basic). 4-Azafluorene was present only in the basic fraction 5. All compounds had identical mass spectra (for more details, see Fig. 3).

Another application is given in Fig. 4. Phthalates had to be quantified in an indoor air sample. Due to cooking activities, the sample contained extremely high amounts of fatty acids, which interfered with the analysis. However, they could be removed with the basic-buffered column, and the remaining compounds were mainly phthalates. Furthermore, the recovery of aliphatic acids in the most polar fractions after prefractionation on acidic-buffered silica was considerably improved, compared with non-treated silica[4].

Based on the experience so far, it can be concluded that prefractionation in

accordance with both polarity and acidity is a very useful tool for compound identification in a complex mixture.

ACKNOWLEDGEMENT

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