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Determination of polycyclic aromatic hydrocarbons in soil samples by micellar electrokinetic capillary chromatography with photodiode-array detection

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Abstract

The reliable quantification even of trace amounts of polycyclic aromatic hydrocarbons (PAHs) is of great concern in environmental and also in medical analysis. PAHs are typically small, uncharged, hydrophobic molecules which do not dissolve well in water. Several methods were investigated and compared for the determination of such substances by capillary electrophoresis, including systems where the analytes are provided with a charge (tetraalkylammonium ions) via solvophobic interaction and systems based on micellar electrokinetic capillary electrophoresis (MECC) using sodium dodecyl sulphate (SDS) and cetyltrimethylammonium bromide as micelle-forming agents. Diode-array detection permitted the positive identification of the separated pure substances via their prerecorded UV–Vis spectra. By using an aqueous–organic electrophoresis buffer [8.5 m*M* borate, 85 m*M* SDS, 50% (v/v) acetonitrile, pH 9.9], a mixture of seven standard PAHs could be separated and quantified within 10 min. The detection limit was 10 pg. The calibration graph was linear over five orders of magnitude. Compared with the chromatographic analysis used so far, the MECC method is faster, has a higher mass sensitivity and requires a smaller sample volume. The method was used to quantify the PAH content of soil samples (heath sand) deliberately contaminated with a mixture of standard PAHs and with machine oil. Two PAHs (anthracene and chrysene) could be determined in samples collected during a biological soil decontamination process.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) contain at least two aromatic rings in their basic structure. Nowadays they are released in large amounts into the environment when oil, coal, wood and related energy sources are converted. Adhering to dust particles, they are then ubiquitously distributed and as hydrophobic substances are enriched in the soil or on entering the food chain, in the fatty tissue of humans and animals. Although less toxic than, e.g., benzene, a number of PAHs have been established as carcinogenic and mutagenic. These potential hazards call for reliable and sensitive PAH determinations in soil and water samples and also in blood and tissue specimens.

Although the total number of PAHs is large, certain of them have been named by governmental agencies such as the US Environmental Protection Agency (EPA) (sixteen PAHs) as indicators of PAH contamination in general. A requirement for soil decontamination will be

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based on the analysis of these sixteen EPA PAHs.

Currently, the determination of these substances is dominated by chromatographic methods such as reversed-phase HPLC [1–8]. Capillary electrophoresis (CE) has become a serious competitor to HPLC in various analytical fields. PAHs, however, appear to be singularly unsuited to CE analysis. In CE the analytes are separated based on differences in their migration velocity in an electrical field. Aqueous buffers are used. Typical PAHs such as anthracene, phenanthrene, fluoranthene and benz[*a*]anthracene are neutral, non-ionizable molecules, of similar hydrophobicity, which do not dissolve well in water in large amounts. The smallness of the required sample, however, together with the high separation power and mass sensitivity, which are inheritant to CE methods, would extend the scope of PAH analysis, e.g., to single cells, airborne water droplets or the determination of PAH metabolites produced by humans or PAH-degrading bacteria.

Only a few approaches to the determination of PAHs by CE have been reported. In one type the analytes are supplied with a charge by forced complex formation with tetraalkylammonium ions and then separated by conventional capillary zone electrophoresis [9,10]. Aqueous–organic (acetonitrile–water) media with various water contents are used as electrophoresis buffers in this approach. More common is separation by micellar electrokinetic capillary chromatography (MECC), often in the presence of sodium dodecyl sulphate (SDS) as a micelle-forming agent and a second migration modifier such as γ-cyclodextrin or methanol [11–15]. Investigations have so far been restricted to the analysis of standard solutions [16]. By using conventional UV detection, a detection limit in the femtomole range is usually attainable, and by using the native UV and fluorescence properties of the PAH for detection, even subattamole levels may be reached [10].

Both UV and fluorescence detection are unspecific. Substances are identified mainly by their migration times. Although this is highly informative in the case of reference or standard samples, it is often not sufficient in the case of real environmental or pharmaceutical samples. Here components of the sample matrix may influence the migration of the analyte to an extent where reliable identification is no longer possible by migration time alone. In addition, often not only the original PAHs are of interest, but also their metabolites, which also constitute highly active carcinogens. Since the metabolic pathways are at present not fully known, little information is available on the exact nature of these derivatives. Specific information on the analytes may be obtained by a coupled technique such as CE–MS, but also by using diode-array detector, which in comparison is a fairly simple and inexpensive alternative. With diode-array detection the entire UV–Vis spectrum of each analyte becomes available and thus enables assumptions to be made concerning the molecular structure.

In this paper, a method is described for the CE determination of PAHs in soil samples with the use of diode-array detection for the on-column identification of the individual substances.

2. Experimental

2.1. Chemicals

Sodium dodecyl sulphate (SDS) was obtained from Serva (Heidelberg, Germany), cetyltrimethylammonium bromide (CTAB) from Lancaster (Morecambe, UK) and boric acid from Merck (Darmstadt, Germany).

2.2. Standards

The PAH standard used contained seven PAHs (anthracene, benzo[*a*]pyrene, chrysene, fluoranthene, fluorene, phenanthrene and pyrene) at a concentration of 1 mg/ml each. The solvent was acetonitrile.

A mixture of machine oils was obtained from a garage in Hannover, Germany.

2.3. Capillary electrophoresis

CE was performed on a Hewlett-Packard 3D-CE instrument. For data collection, data analysis, spectral identification and system control, HP 3D-CE (Rev. A. 01.02.) software was used. Detection was by measurement of UV–Vis absorbance with a photodiode-array detector (total range, 190–690 nm; range used, 190–350 nm). Capillaries were obtained from Hewlett-Packard (Böblingen, Germany) or CS-Chromatographie Service (Langerwehe, Germany). Unless indicated otherwise, 8.5 m*M* borate buffer (pH 9.9) containing 85 m*M* SDS and 50% (v/v) acetonitrile was used as the electrophoresis buffer. The capillary dimensions were 28 cm (from inlet to the detector) × 50 μm I.D., a voltage of 30 kV was applied and the capillaries were thermostated at 20°C. Pressure injection (30 mbar, 5 s) was used. After each analysis, the capillaries were regenerated with consecutive washes with 0.1 *M* NaOH (1 min) and electrophoresis buffer (2 min).

The buffer in the outlet vial was exchanged after each run to improve the reproducibility. After each injection the capillary was briefly dipped in a second buffer vial to remove all traces of sample from the outer capillary wall.

2.4. Contamination of soil samples

A 10 g amount of sand (heath) was wetted with 0.5 ml of a solution containing the seven standard PAHs each at a concentration of 1 mg/ml.

A 30 g amount of sand (heath) was contaminated with 1 ml of spent machine oil. Extraction was carried out after 2 h.

2.5. Extraction of soil samples

In the standard procedure, 0.5 g of soil was extracted with 6 ml cyclohexane with vigorous shaking (15 min), washed with an additional 4 ml of cyclohexane, evaporated to dryness and the residue dissolved in 0.5 ml of acetonitrile.

After contamination with machine oil, 10 ml of cyclohexane were used for extraction of 10 g soil (10 ml for washing). The dried residue was dissolved in 2.5 ml of acetonitrile.

2.6. Biological soil decontamination

Samples from a biological soil decontamination process were kindly provided by D. Brinkmann (Institut für Technische Chemie, University of Hannover, Germany). In this process, the water content of the PAH-containing soils is increased to a point where the resulting slurries can be handled in an airlift or rotating drum reactor [17,18]. The soil decontamination is then performed with a mixed culture of aerobic bacteria capable of PAH digestion. At this point it is not clear whether the PAHs are completely converted into CO_2 and H_2O or whether more complex (and putatively still harmful) metabolites are produced. PAH-determination is carried out by reversed-phase chromatography (column dimensions, 100 mm × 4.6 mm I.D.; stationary phase, adsorbosphere C_{18} (Alltech), particle diameter 3 μm; mobile phase, acetonitrile–water (86.4:13.6); flow-rate, 1.26 ml/min; detection wavelength, 254 nm).

3. Results and discussion

The separation of electrically neutral molecules of similar size and hydrophobicity by CE is difficult. Unless the analytes can somehow be converted into charged species, MECC may often constitute the only way to effect such an analysis.

Since charged molecules can often be separated in free solution whereas MECC requires complex electrolysis buffers, first we tried the solvophobic method introduced by Walbroehl and Jorgenson [9] for PAH separation. In this method, the hydrophobic analytes are forced to form complexes with tetraalkylammonium ions. However, neither the commonly used tetrahexyl-

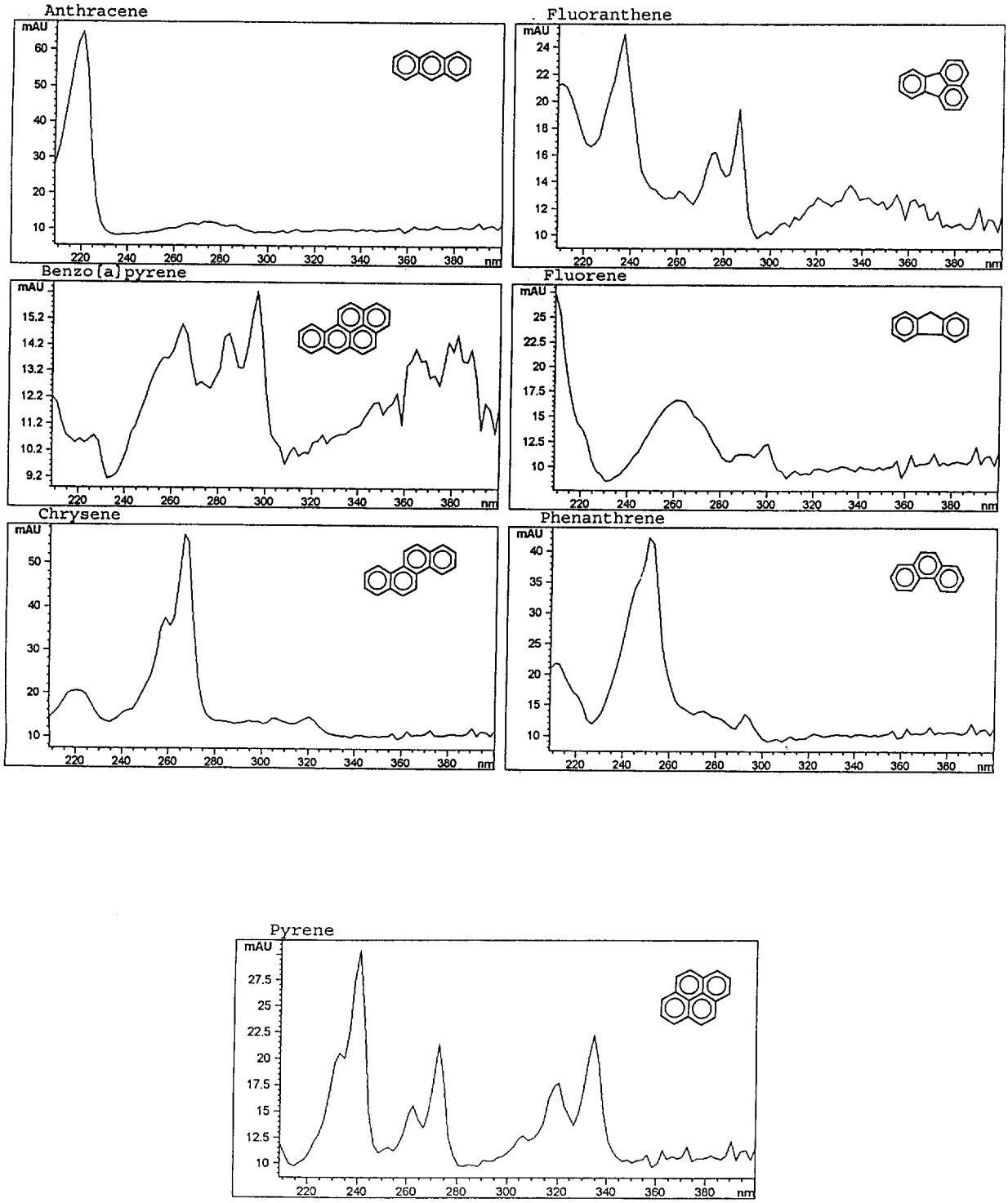


Fig. 1. UV–Vis spectra of the standard PAHs.

ammonium ion nor the use of tetraheptylammonium, tetraoctylammonium, tetradodecylammonium, tetraacetylammonium, N-(2-ethylhexyl)trimethylammonium or N-octyltrimethylammonium ions resulted in an applicable method. Acetonitrile–water mixtures of various compositions were investigated and the effect of different modifier concentrations was evaluated, but to no avail. In addition, the bromides used here were difficult to dissolve in the aqueous–organic buffers.

MECC is the classical CE method for the analysis of neutral hydrophobic molecules. The surface charge of the micelles draws them at a characteristic speed towards the corresponding electrode, in the case of SDS this being the anode, i.e., under standard conditions opposite to the electroendosmotic flow (EOF) and away from the detector. Largely governed by their hydrophobicity, the analytes will partition between the hydrophobic interior of the micelles and the bulk of the electrophoresis buffer, where the migration of neutral molecules will in uncoated capillaries be determined by the EOF. As in reversed-phase chromatography, which is governed by similar interactions, the attractiveness of the bulk buffer phase may be enhanced by the addition of an organic modifier such as acetonitrile.

Since the differences in hydrophobicity of the PAHs are not very pronounced, the addition to the electrophoresis buffer of a second substance, such as γ-cyclodextrin, has been suggested, which also interacts actively with the analytes

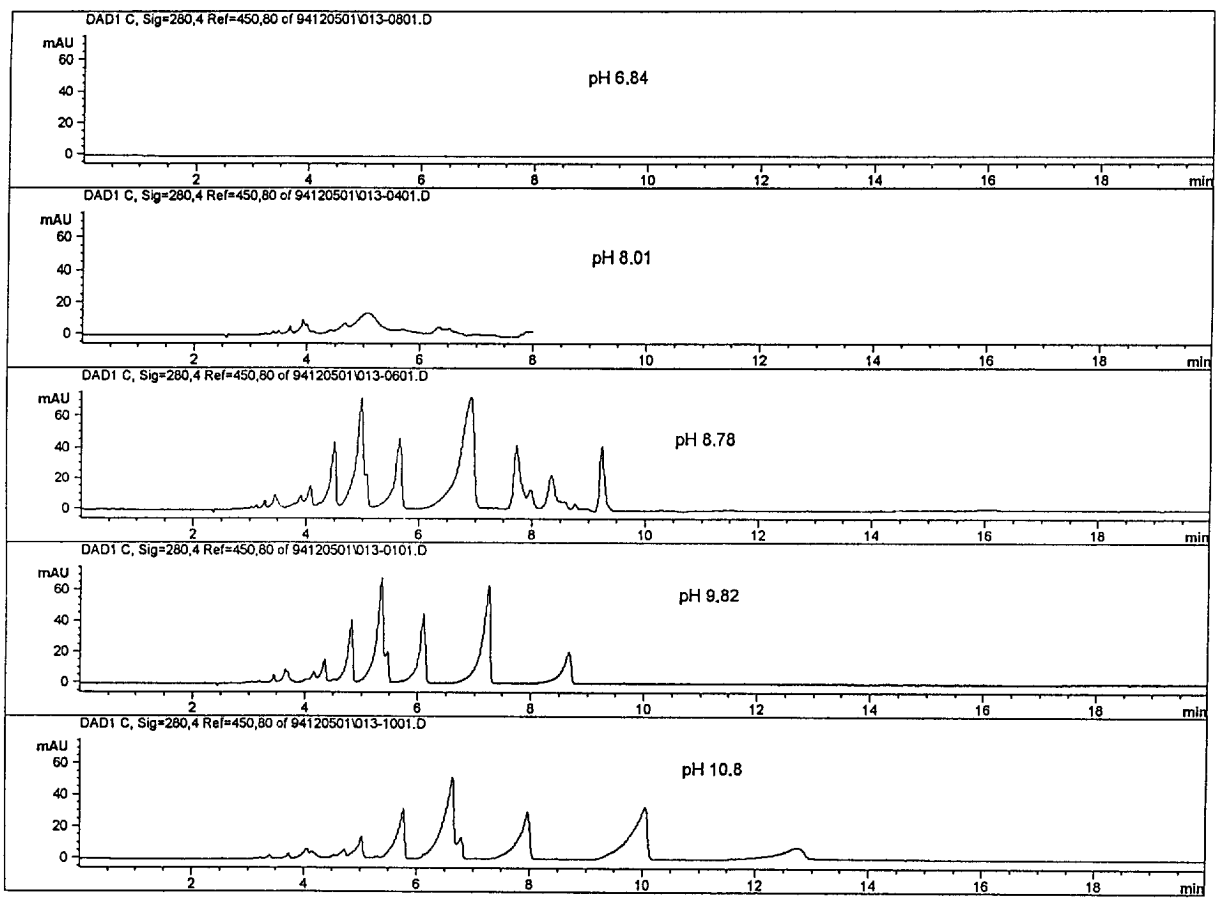


Fig. 2. Influence of buffer pH on the PAH separation.

[14,15]. However, this renders the fine tuning of the analytical conditions difficult and we found the modification of the bulk buffer by addition of acetonitrile to be more suited to our purposes.

3.1. Method development

Capillary dimensions

For method development, a PAH standard mixture was used that contained anthracene, fluorene, phenanthrene, fluoranthene, pyrene, chrysene and benzo[*a*]pyrene in acetonitrile at a concentration of 1 mg/ml each. The UV–Vis spectra of these standard PAHs were compiled into a spectral bank (Fig. 1). Unless indicated otherwise, the capillaries had an I.D. of 50 μm and an effective length (inlet to detector) of 28 cm. A larger I.D. resulted in a lower detection limit owing to the increase in the optical path length. However, as high voltages of up to 30 kV were used, the ensuing radial temperature gradient was detrimental to the resolution in the case of, e.g., a 100 μm capillary. Hewlett-Packard offers the use of bubble cell capillaries to circumvent this problem, but we did not find these capillaries useful in our case. An effective capillary length of 28 cm represents a compromise between the resolution and the duration of an analysis.

Influence of the buffer pH

The influence of the pH of the electrophoresis buffer on the separation is depicted in Fig. 2. At

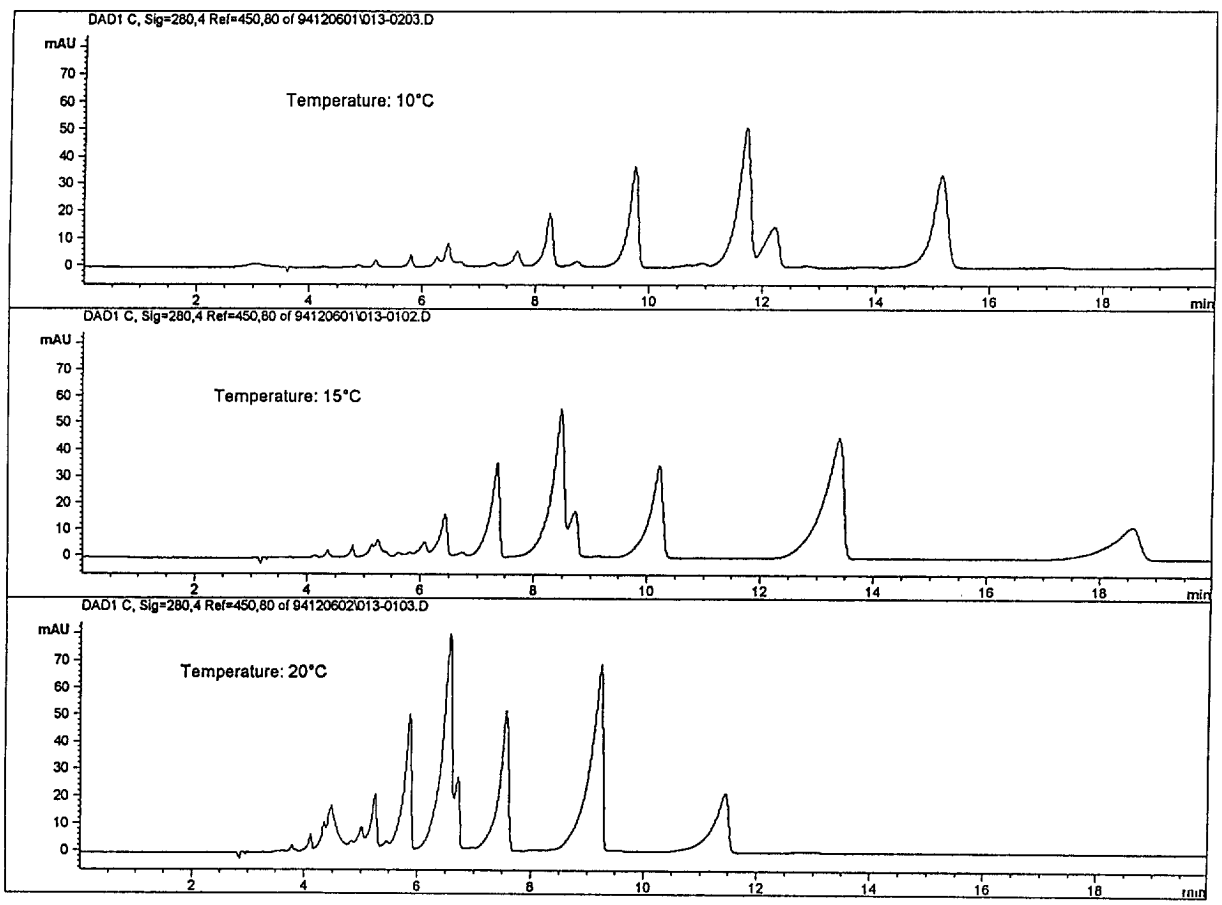
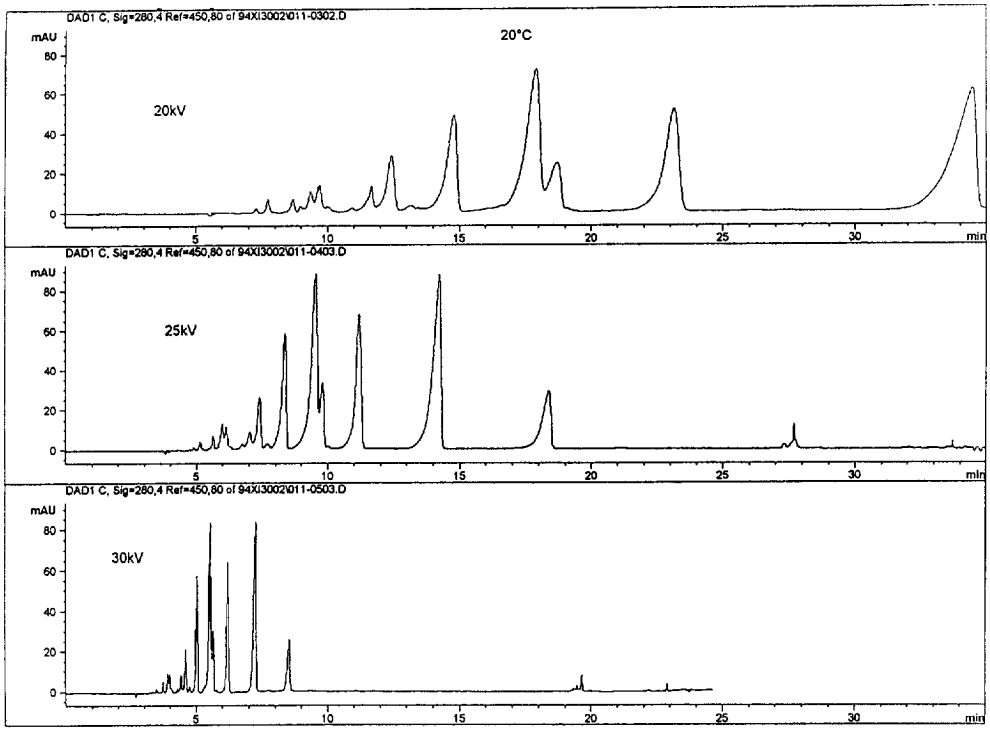


Fig. 3. Influence of temperature on the PAH separation.



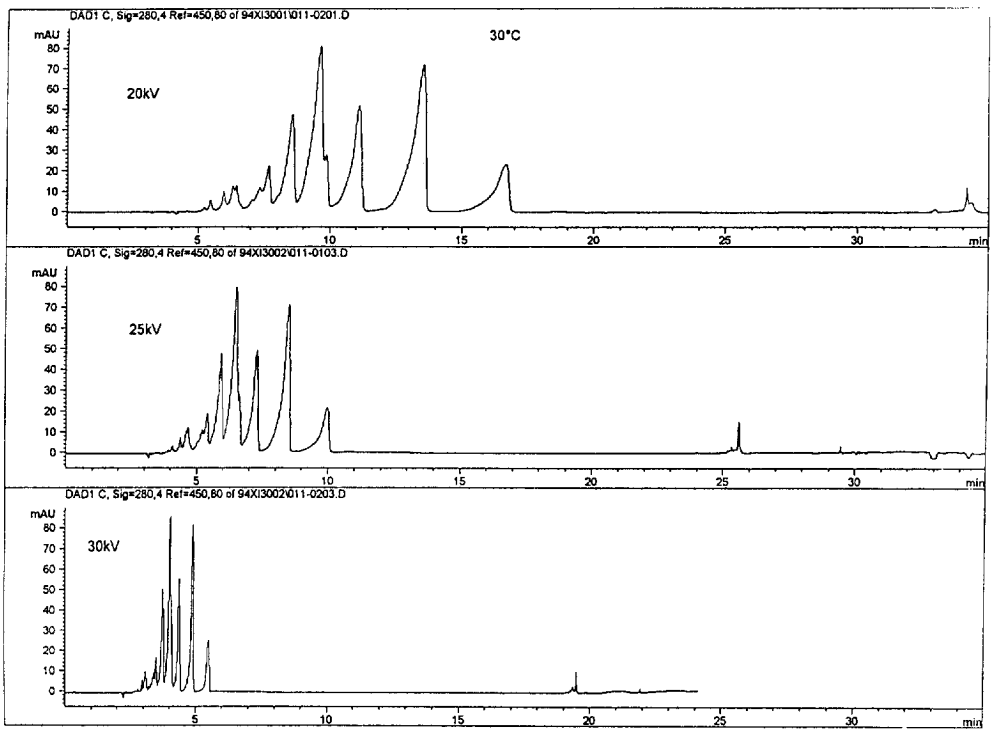


Fig. 4. Influence of applied voltage on the PAH separation.

a pH of 6.5 no signals are detected. Presumably the EOF is not strong enough in this case to counteract the movement of the micellar phase towards the capillary inlet. If a pH of more than 8.0 is applied, the analytes begin to pass the detector, but the separation is not satisfactory. If the pH is increased further, the separation improves to some extent, while the migration time increases with increasing pH. A buffer pH of 9.9 was adopted for standard applications, since it combined good resolution with a comparatively short analysis time. A borate-based buffer was chosen, since this yielded the lowest background absorption in the wavelength range investigated. Compared with a phosphate buffer of similar ionic strength, the electric current and thus the Joule heat produced were lower for a given voltage. With regard to the detrimental effect of heat in general, a low buffer concentration of 8.5 m*M* was used. This, however, calls for an exchange of the buffer in the capillary vials after each NaOH wash, or else its pH and concomitantly the electrophoretic character of the CE system are altered.

Influence of temperature and applied voltage

Temperature and applied voltage also influence the duration and to some extent the resolution of a given separation. The migration time will increase with decreasing temperature (Fig.

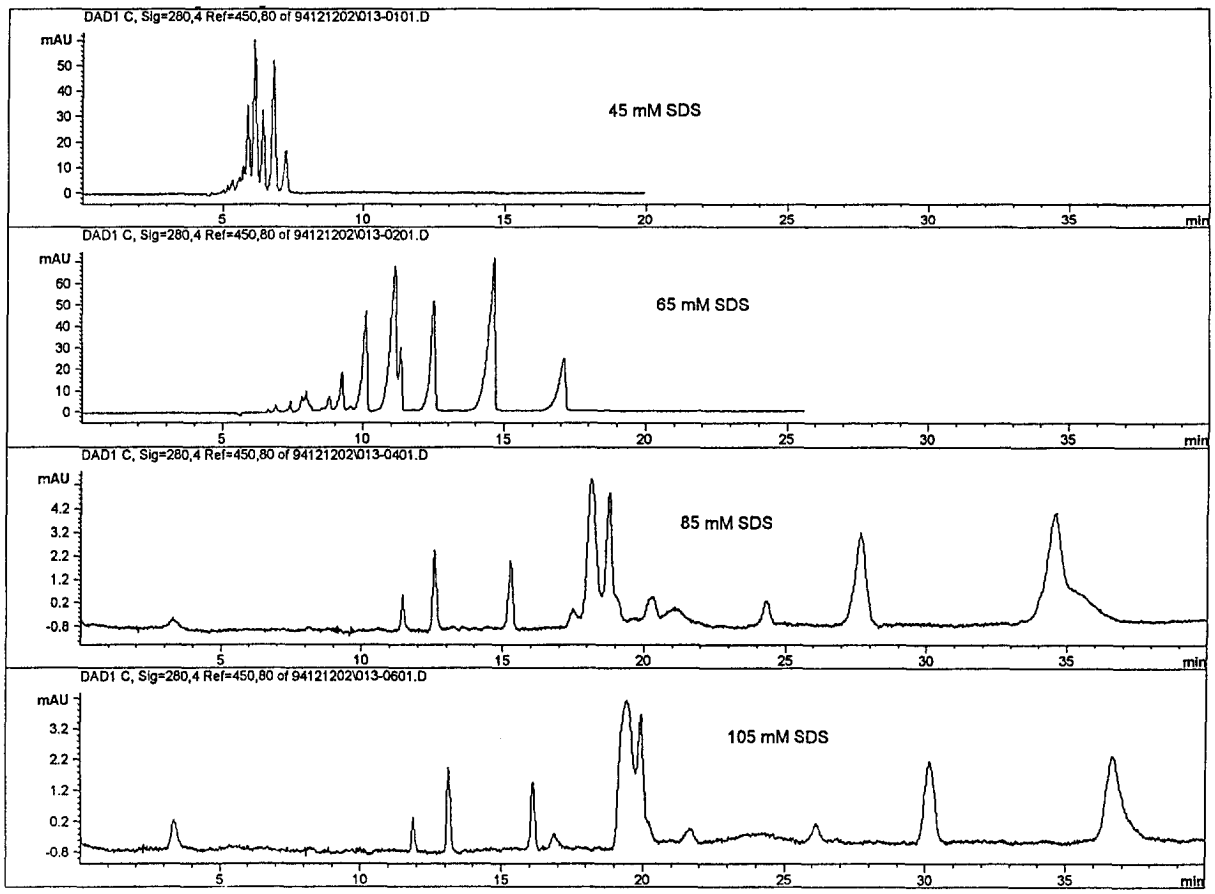


Fig. 5. Influence of SDS concentration on the PAH separation.

3), since the viscosity of the buffer will also increase in that case. Increasing the applied voltage from 20 to 25 and 30 kV for a given temperature reduces the migration time in all cases (Fig. 4). However, at 30°C no baseline separation was found even in those cases, e.g., at 25 kV, where the separation took longer than a similar separation performed at 20°C and 30 kV. A voltage of 30 kV was therefore adopted for standard PAH determinations, while the capillary was thermostated at 20°C. Under these conditions, good resolution of the PAH standard mixture was achieved, with the exception of fluoranthene and pyrene. These two PAHs can, however, still be identified via their UV–Vis spectra and be quantified from the peak height. This is not possible with the reversed-phase chromatographic method used as the reference method for PAH determination.

Influence of micelle and modifier concentration

The influence of the SDS concentration of the PAH separation was investigated using a 40 cm capillary and the above-defined standard conditions. As other workers, we observed an increase in migration time on increasing the SDS concentration, i.e., from 45 to 65, 85 and 105 m*M* [14]. SDS concentrations below the critical micelle concentration were not investigated. The observed increase is caused by the increase in

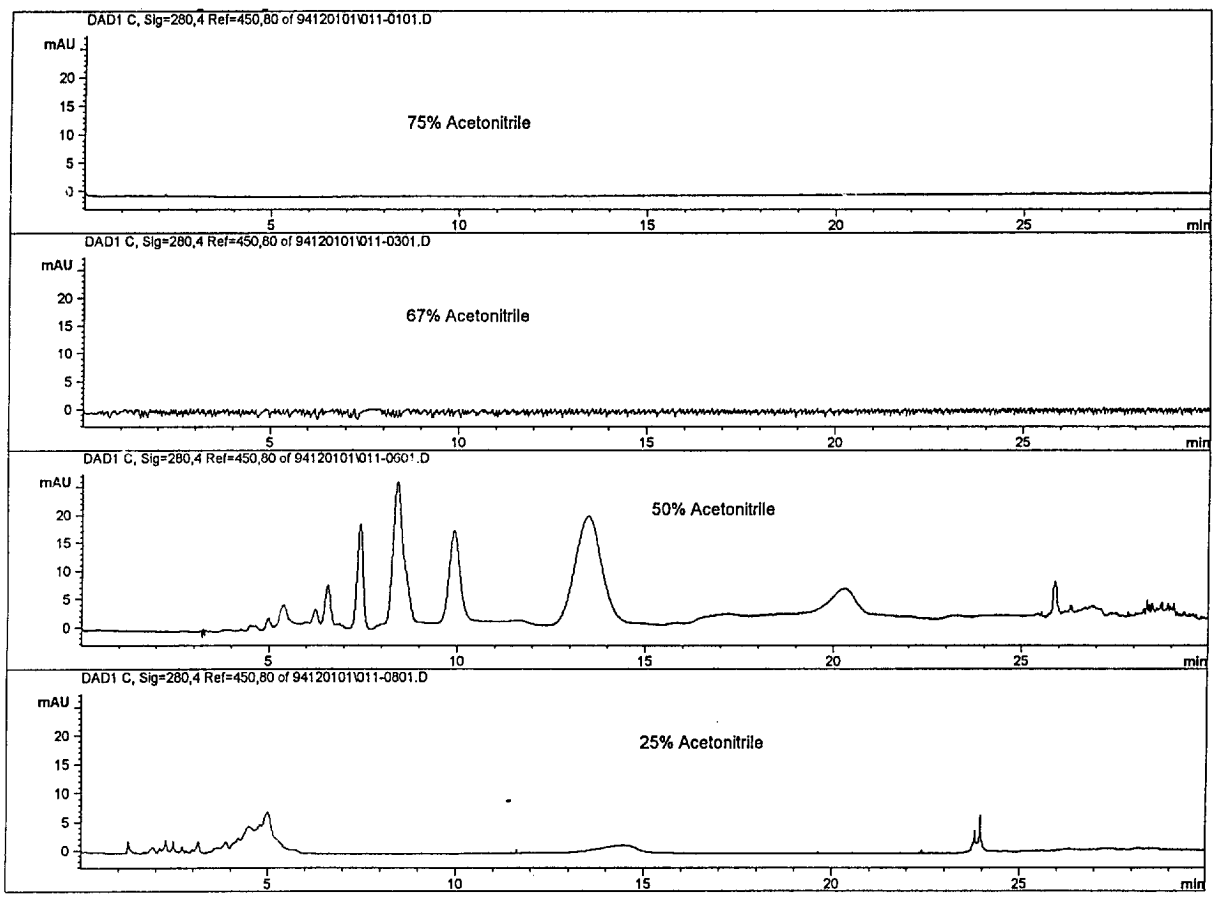


Fig. 6. Influence of acetonitrile concentration on the PAH separation.

micelle concentration. The resolution in terms of a fluoranthene–pyrene separation was best with an SDS concentration of 85 m*M* (Fig. 5).

Similar reasoning led to the addition of 50% acetonitrile to the electrophoresis buffer (Fig. 6). At too low an acetonitrile concentration the solubility of the analytes is low and at too high a concentration micelle formation appears to suffer interference.

3.2. CTAB as micelle forming agent

A similar development led to an alternative method in which CTAB rather than SDS was employed as a micelle-forming agent. The 50 m*M* borate buffer (pH 9.5) used contained 100 m*M* CTAB and 25% acetonitrile. The capillaries had an optimum length of 58 cm and an I.D. of 75 μm. The capillaries were thermostated at 25°C and a voltage of 20 kV was applied. However, with this method the resolution, especially of fluoranthene and pyrene, was inferior to that in the SDS method (Fig. 7).

3.3. Applications

A detection limit of 10 ng/μl, i.e., 10 pg, was established for the developed method. The calibration graph is linear over five orders of magnitude (Fig. 8). An analysis requires less than 10

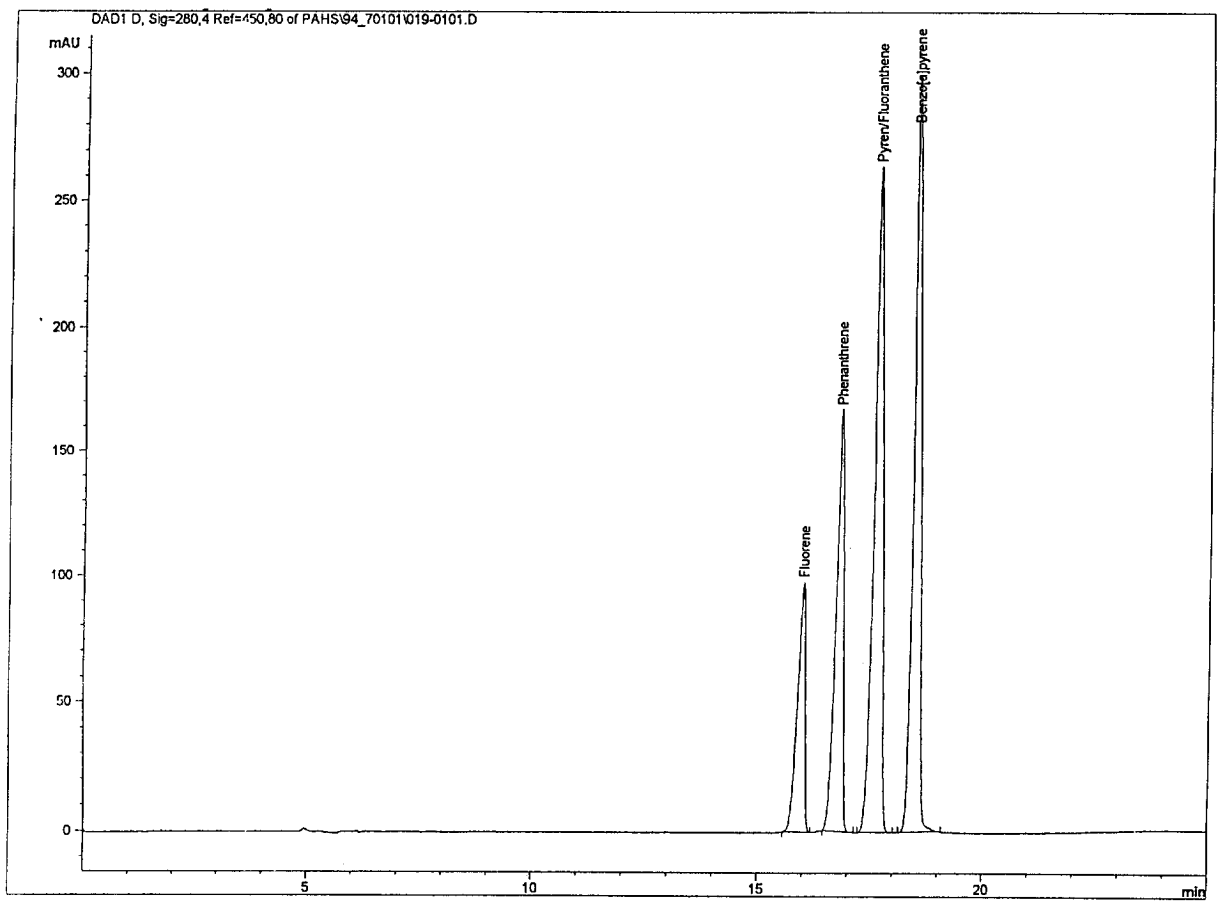
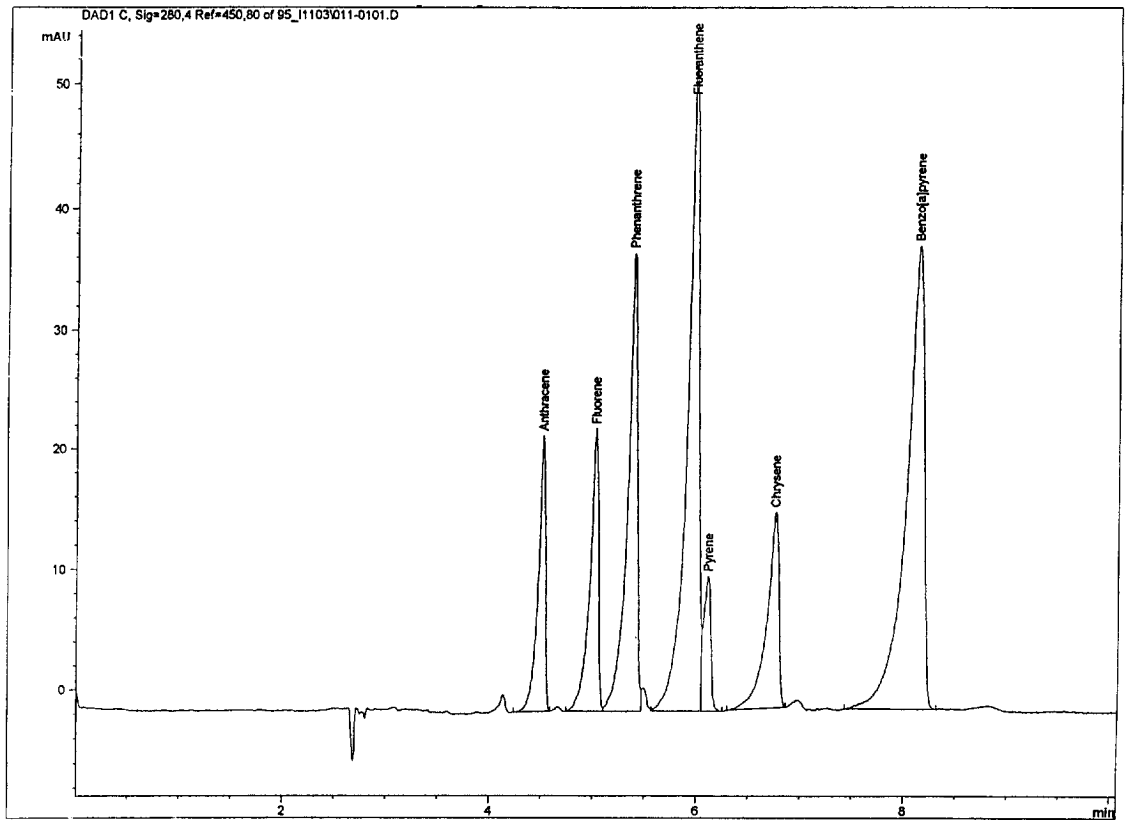


Fig. 7. Separation of a five PAH standard mixture by the CTAB method.



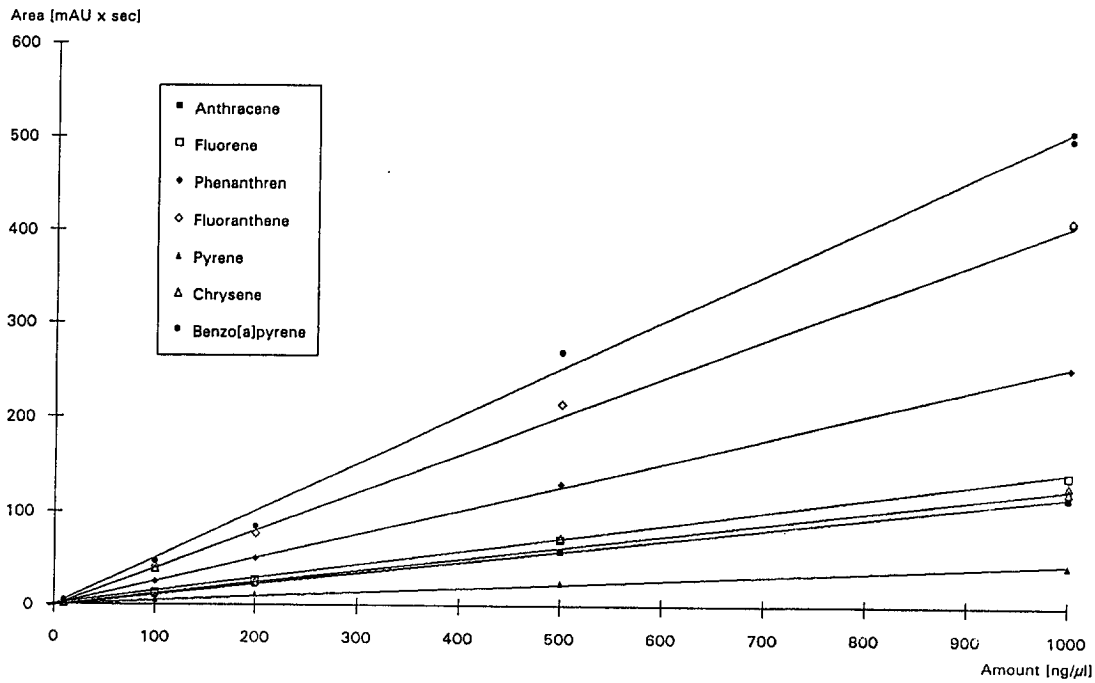
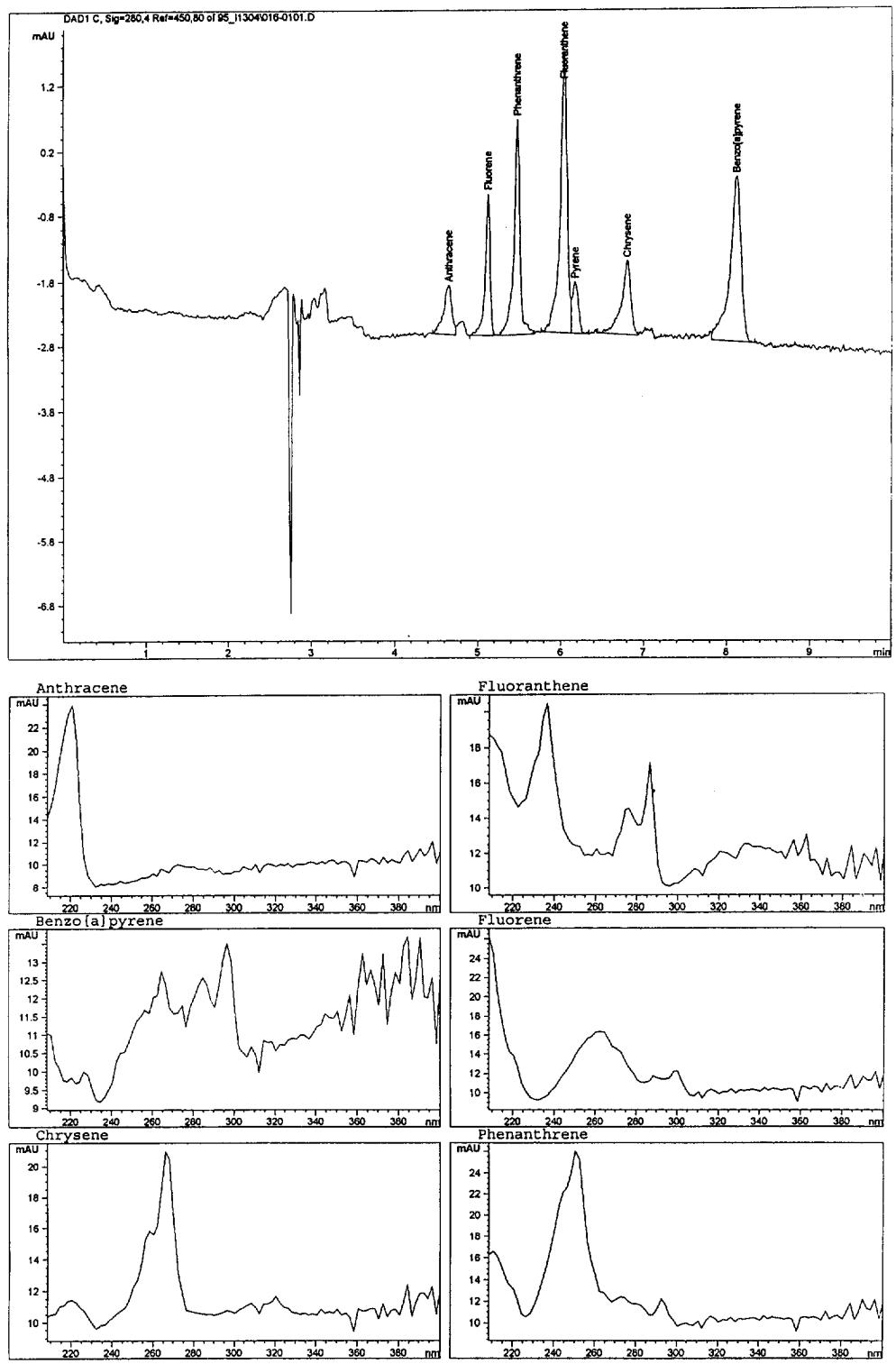


Fig. 8. Calibration graph for the standard PAHs (SDS method).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mAU
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Fig. 9.

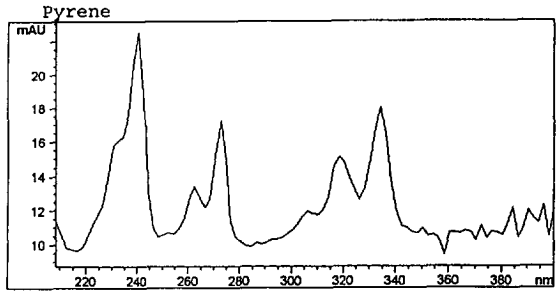

Fig. 9. Analysis of a soil sample contaminated with a PAH mixture. The spectra assigned to the peaks are also given.

min. The analytes are well separated and can be positively identified by comparison with their stored reference UV–Vis spectra. To increase the reproducibility of an analysis, the use of an internal standard, most conveniently the acetonitrile peak, is recommended. In comparison, 40 min are required for an analysis using the conventional RP-HPLC method. There the detection limit is 0.5 ng and no separation of fluoranthene and pyrene is possible.

Analysis of a soil sample contaminated by a PAH mixture

Ultimately, the developed method is to be used to monitor the success of the decontamination of PAH-polluted soils by PAH-degrading bacteria. To simulate such an analysis, a "soil sample", i.e., 10 g of sand (heath), was "contaminated" with 0.5 ml of a solution containing seven PAHs each at a concentration of 1 mg/ml. The standard extraction procedure that had

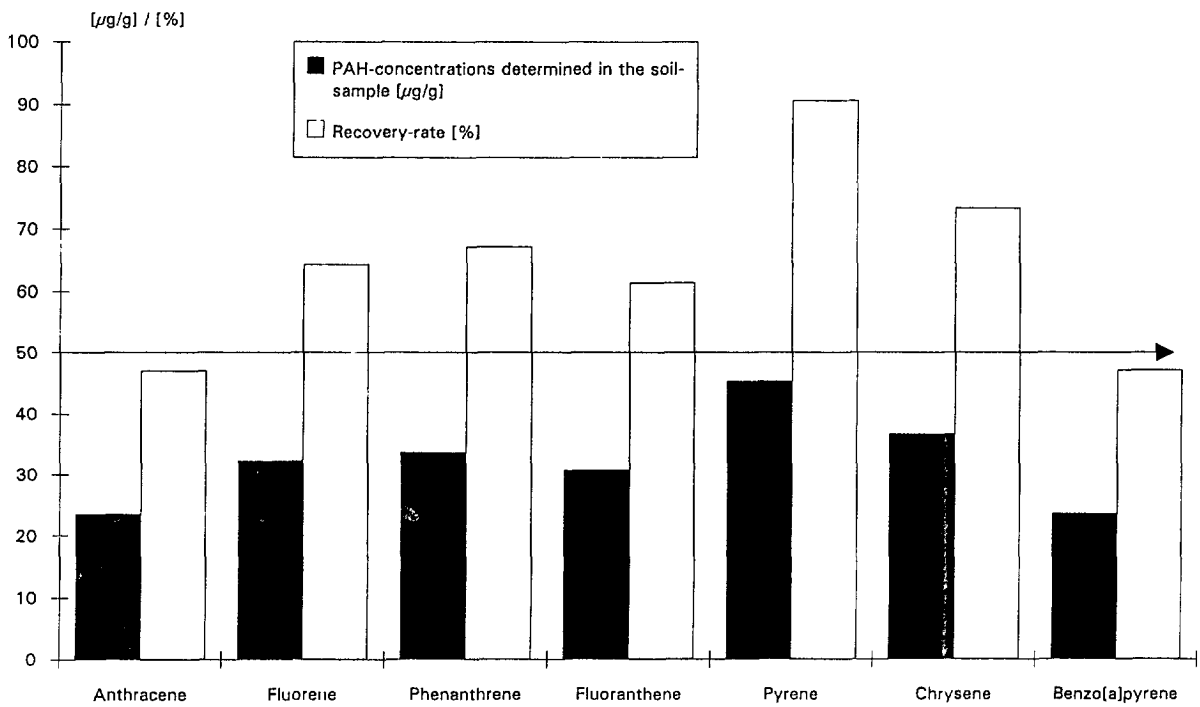

Fig. 10. Quantification of the various PAHs identified in the soil sample compared with the original amount.

originally been developed for the RPC analysis was used to prepare the sample for analysis. The results are depicted in Fig. 9. All substances are identified by their spectra. The quantification is unsatisfactory, as only 45–90% of the original amount is recovered (Fig. 10). Further efforts need to be directed towards the optimization of the extraction procedure.

Analysis of a soil sample contaminated by machine oil

Soil contamination was also simulated with spent machine oil. A 30 g amount of sand (heath) was contaminated with 1 ml of spent machine oil. After 2 h, extraction (by a modified procedure) and MECC analysis were carried out. The MECC analysis yielded reproducible results, an example being given in Fig. 11. However, no PAH identification was possible. Presumably, either no pure substances were separated or the PAHs considered so far were not present in this sample.

Analysis of samples collected during a biological soil decontamination process

In the samples collected during a biological soil decontamination process, chrysene and anthracene could be identified and determined (Fig. 12).

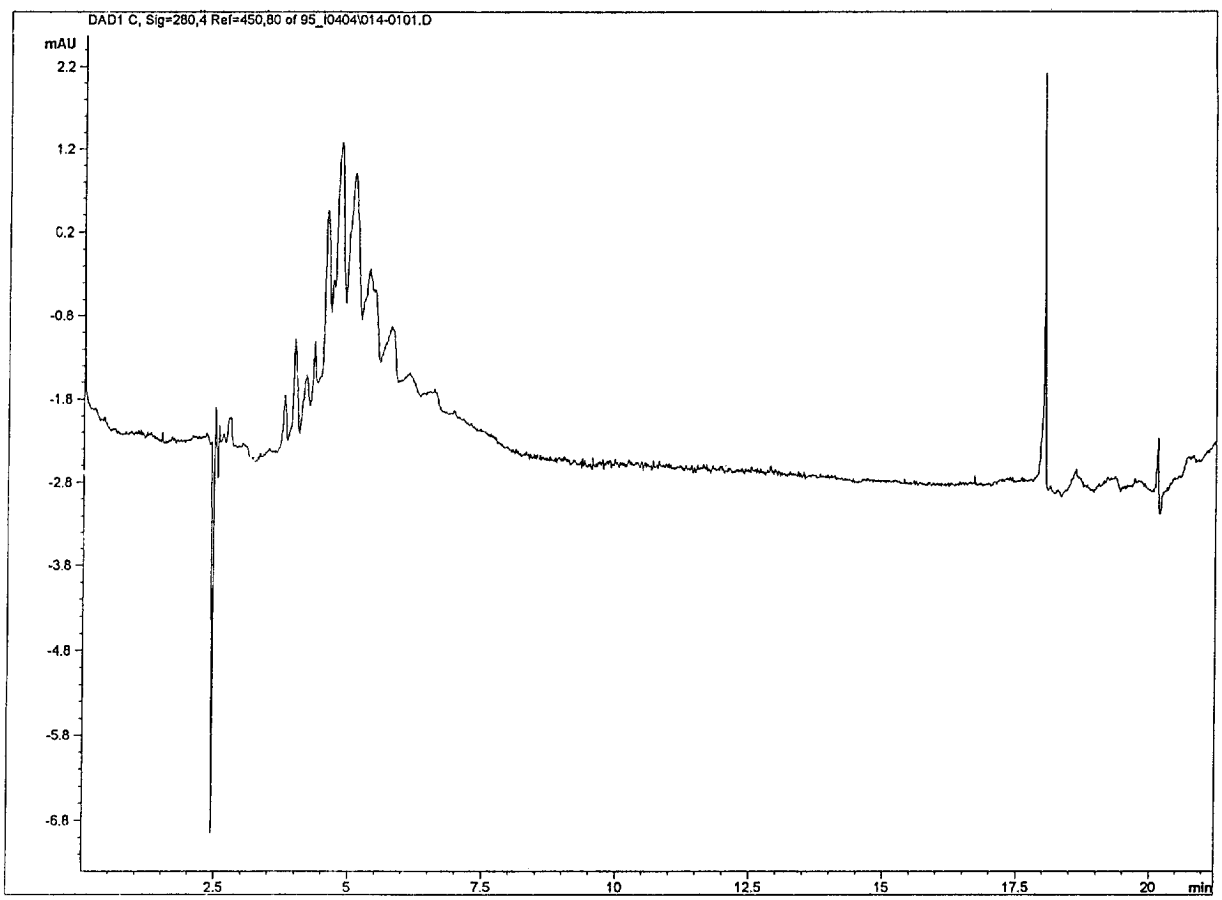

Fig. 11. Analysis of a soil sample contaminated with machine oil.

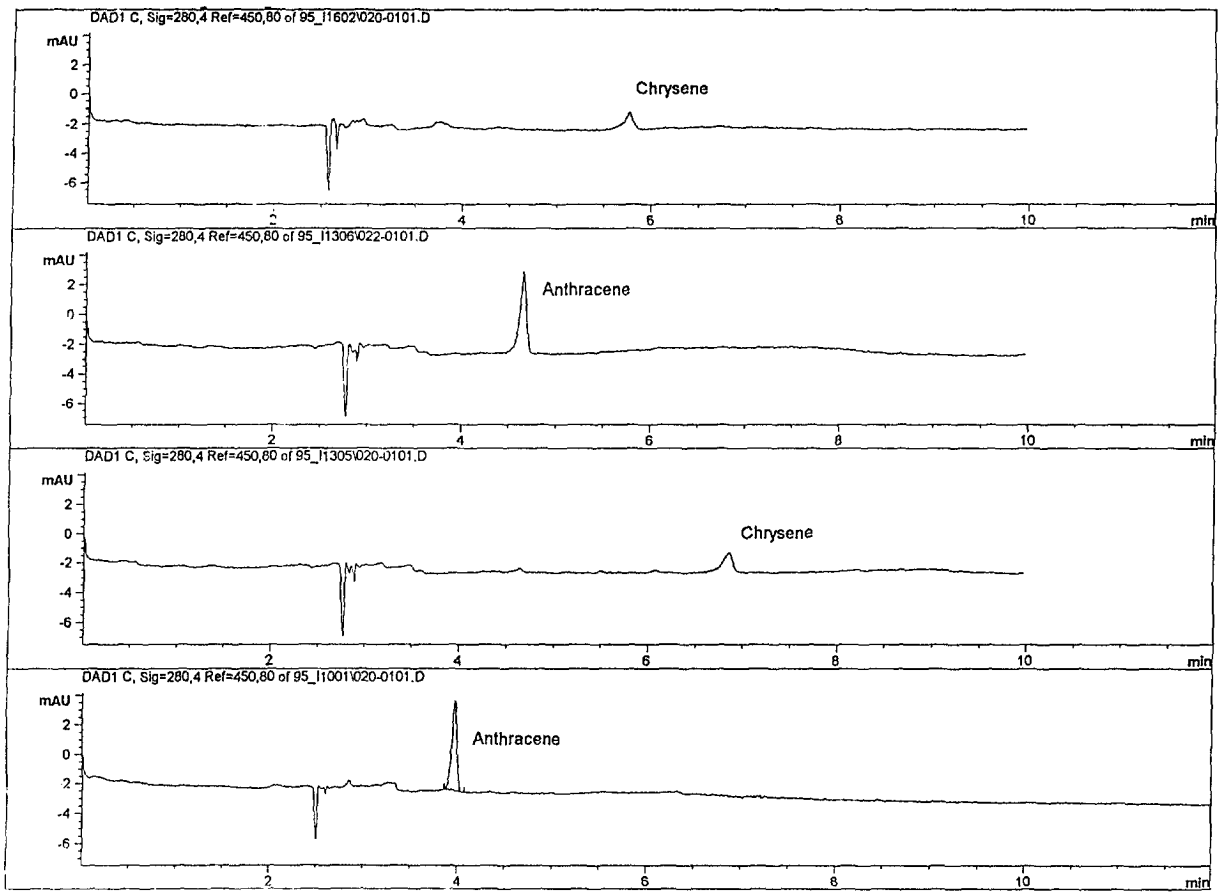

Fig. 12. Analysis of soil samples collected during a biological soil decontamination procedure.

Acknowledgements

Hewlett-Packard kindly made a CE system with a photodiode-array detector available to us for the duration of these experiments. Mr Dirk Brinkmann's help in procuring the samples of contaminated soils is gratefully acknowledged.

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