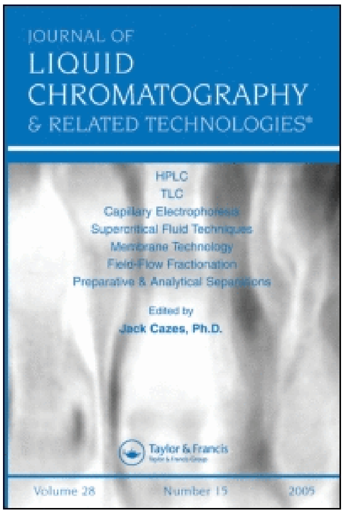

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DIRECT EXTRACTION AND SEPARATION OF SOME POLYNUCLEAR AROMATIC HYDROCARBONS (PAHs) FROM COMPLEX MIXTURES (SEWAGE SLUDGES) BY COUNTERCURRENT CHROMATOGRAPHY

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ABSTRACT

The present work is the first attempt to use countercurrent chromatography for xenobiotics extraction and separation from complex natural matrices without sample pretreatment. A procedure has been developed for the direct extraction and separation of some PAHs from a sewage sludge medium being in fact a concentrated suspension. The sewage sludge and aqueous solutions containing the trace amounts (0.01-10 μg/mL) of PAHs were used as the stationary phase in the column whereas organic solvents (n-heptane, dichloromethane) or their mixture were used as mobile phase.

Taking pyrene as an example it has been shown that the recovery of PAHs can be quantitative. When no PAHs was added to the

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sewage sludge, pyrene was found in the sample investigated at the level of 0.24 μg/mL.

The possibility of the subsequent separation of naphthalene, pyrene, and benzo(g,h,i)perylene from the sewage sludge medium also has been demonstrated.

INTRODUCTION

Organic micropolluantes (xenobiotics) are widespread in the environment as a result of human activities (industry, agriculture, and traffic). In recent years a great number of studies have been published dealing with different analytical techniques used for determining these trace compounds in various compartments of biosphere. Estimating the state of contamination of sewage sludges is one of the most important problems which is currently attracting an interest of investigators working in the field of ecology and environmental chemistry.

In general, sewage sludges being in fact a more or less concentrated suspension can be divided into the following principal groups: organic hydrophilic, oil (hydrophilic and hydrophobic), mineral (hydrophilic and hydrophobic), and fibrous.[1] Since we are considering the organic micropolluants, two groups (organic and oil) are most interesting.

Sewage sludges are known to contain various contaminants, some of them are given in Table 1. Corresponding methods of separation are also presented. Polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and phenols are toxic, most widespread and, consequently, most dangerous among the products enumerated. Qualitative and quantitative analysis of these compounds is the aim of numerous studies.[2-9] Sewage sludge is a complex matrix containing solid suspension particles and, in major cases, the analytical procedures proposed are multistage, time-consuming, and require different methods of separation and determination.

A procedure for simultaneous determination of PAHs and PCBs in the sewage sludges was proposed.[6] First, a sample was saponified for 2 hours at 70°C in 2 mol/L KOH in methanol. After liquid-liquid extraction with hexane the non polar phase was reduced in volume and further cleaned on a double layer column consisting of silica gel and aluminium oxide. Then, PCBs and PAHs were eluted by hexane-dichloromethane (9:1) and hexane-dichloromethane (4:1) mixtures, respectively. After preconcentration and separation PCBs and PAHs were determined by high resolution gas chromatography with different types of detectors. It should be noted that the procedure proposed is rather complicated.

Table 1

Some Methods for Estimating the State of Contamination of Sewage Sludges

Compounds	Methods for Separation (Recovery)	References
Polyaromatic hydrocarbons (PAHs)	Supercritical fluid extraction (SFE)	[2-4]
	Ultrasonic extraction, Soxhlet extraction	[5]
	Liquid-liquid extraction followed by adsorption chromatography	[6]
Polychlorinated biphenyls (PCBs)	Liquid-liquid extraction followed by adsorption chromatography	[6]
	Subcritical water extraction	[7]
Phenols	SFE	[3,4,8]
	Liquid-liquid and Soxhlet extraction followed by capillary gas chromatography	[9]
Atrazine	Liquid-liquid extraction followed by gas chromatography	[10]
Detergent-derived fluorescent whitening agents	Liquid chromatography	[11]
Sulphonated aliphatic and aromatic surfactants	Ion-pair SFE	[12]

In some cases supercritical fluid extraction (SFE) allows one to separate xenobiotics directly from natural samples using carbon dioxide.[2] However, it requires different modifiers and high temperature to reach a satisfactory product yield. Soxhlet extraction also makes it possible to separate PAHs and other pollutants without preliminary sample preparation but the procedure is very time-consuming (15-50 hours).

Countercurrent chromatography (CCC) can be a very attractive technique for preconcentration and separation of different xenobiotics from complex natural samples containing suspended particles. CCC is based on the retention of one phase (stationary) of a two-phase liquid system in a rotating coiled column under the action of centrifugal forces while the other liquid phase (mobile) is being continuously pumped through.[13]

An important distinguishing feature of CCC as a chromatographic method is the absence of an adsorptive matrix for retaining the stationary phase. This feature determines the main advantages of the method, such as the absence of solute loss due to interaction with the sorbent matrix, a variety of two-phase liquid systems may be used, easy change from one partition system to another, the possibility to change a volume of the sample solution from 0.1 to 1000 mL or more, and a high preparation capacity provided by a high ratio (up to 0.9) of the liquid stationary phase volume and the total column volume (this ratio is much higher than that for the stationary solid phase used in HPLC).[13,14] It should be noted that the problem of column packing is also eliminated in CCC and the stationary phase is relatively inexpensive.

A few devices providing retention of the stationary phase in the field of mass forces in the absence of a solid support have been suggested. Among the various possible designs, the planetary centrifuge retains the liquid stationary phase effectively and enables the fastest and most efficient separation to be achieved. A column (or a column unit) of a certain configuration rotates around its axis and simultaneously revolves around the central axis of the device with the aid of a planetary gear.

We are the first who are going to apply CCC to the analysis of complex natural suspended samples like sewage sludges.

EXPERIMENTAL

Apparatus

The separation was performed on a CCC device of J-type (Ito's classification)[13] consisting of a planet centrifuge with a horizontal column drum equipped by three multilayer coil columns (Pharma-Tech Research Corp., USA, Model CCC-2000), a chromatographic pump (Varian, France), and a UV-detector (Varian, France). Only one coil column made of a Teflon tube with an inner diameter of 1.85 mm was used for our experiment. The total inner capacity of the column was 14 mL. β-Parameter ($\beta = r/R$, where r and R are rotation and revolution radii, respectively) is not a constant value for multilayer columns. In our case β varied from 0.38 (internal layer) to 0.53 (external layer). The mobile phase flow rate (F) was equal to 1.0 mL/min. The rotation speed was 900 rpm. A dynamic mixing chamber (Gilson, France) and another chromatographic pump (Gilson, France) were used for the post-column dilution of the effluent with isopropanol.

Samples and Reagents

Samples investigated were taken at the sewage disposal station of Paris. All chemicals used were analytical grade reagents.

CCC Procedure

For the preconcentration and separation of PAHs, we used the following chromatographic procedure. Before commencing the CCC experiment, the organic solvents were stirred with water and brought into equilibrium, after which the organic solvents were used as the mobile phase. The spiral column in the stationary mode was filled with the organic phase, then, the sample (2-5 mL) was introduced into the column. After it, while the column was rotated, the organic phase was fed to its inlet again. The mass force field which arose during rotation made it possible to retain the stationary phase (in our case, an aqueous solution with suspended particles) in the column while the mobile phase was continuously pumped through. The pumping rate was 1.0 mL/min.

Detection of PAHs

In order to reduce the background level, isopropanol was continuously added to the column effluent before the UV-detection of PAHs. The pumping rate of isopropanol was 0.5 mL/min. Two flows were mixed in the dynamic mixing chamber. Then, the absorbance of the resulting flow was monitored at λ= 254 nm.

Fractions of the detector effluent (7-10 mL each) collected for further HPLC analysis were evaporated after it was dissolved in acetonitrile (2 mL). Then, the samples were introduced into Chrompack column. The flow rate was 0.7 mL/min. Conditions for the fluorescence detection of PAHs were the following: excitation at 295 nm; emission at 380 nm.

RESULTS AND DISCUSSION

Retaining the Suspension in a Rotating Coiled Column

As it has been mentioned above, a sewage sludge medium is, in fact, a more or less concentrated suspension. Up to this point CCC has been utilised for the analysis or purification of liquid homogeneous samples. We have demonstrated that the retention of an aqueous sample containing solid particles is possible. No carryover of the stationary phase (the sewage sludge) has been observed, while the organic mobile phase used is being continuously pumped through.

It should be noted, that a fixed volume of the stationary phase (2-5 mL) was introduced into the column, whereas it is principally possible to retain up to 8 mL of the aqueous sample under the given experimental conditions.

A n-heptane-dichloromethane mixture has been used as the mobile phase; the content of dichloromethane in the mixture is being varied from 0 to 20%. The density difference between two phases is known[13,15] to be the most important moving force of the stationary phase retention in CCC. If dichloromethane (density $\rho = 1.33$ g/cm^3) is added to n-heptane ($\rho = 0.69$ g/cm^3), the mobile phase becomes heavier and the density difference between organic and aqueous phases decreases.

The mixture containing 20% of dichloromethane ($\rho = 0.81$ g/cm^3) remains suitable for the stationary phase retention under the given conditions. However, when the mobile phase contains 30% of dichloromethane ($\rho = 0.88$ g/cm^3), the carryover of the stationary phase may occur. If it is needed, the retention in such systems can be improved by increasing the rotation speed and by using the columns with larger total volume.

Recovery of Pyrene from a Sewage Sludge Medium

A saturated aqueous solution of pyrene (0.25 μg/mL), the sewage sludge, and their mixtures (Table 2) were used as the stationary phase, whereas the n-heptane-dichloromethane (4:1) mixture was used as the mobile phase. The effluent was monitored by the UV-detector. The peaks obtained differ in height, however, in all cases their shapes are similar. The elution of pyrene and other detected components of the samples begins immediately after a volume of the eluate equal to the volume of the mobile phase in the column has passed through (Figure 1).

A fraction of the detector effluent (8 mL) containing the eluted components was collected for further HPLC analysis with fluorescence detection so that the recovery yield of pyrene could be determined easily. According to the

Table 2

Results of the Fluorescence Determination of Pyrene in the Effluent

Sample Injected in the CCC Column	Pyrene Contents
Aqueous solution of pyrene, 2 mL	0.50 μg
Sewage sludge, 2 mL	0.48 μg
Aqueous solution of pyrene, 2 mL + sewage sludge, 2mL	1.00 μg
Sewage sludge, 4 mL	0.98 μg
Blank sample, 4 mL of water	<0.02 μg

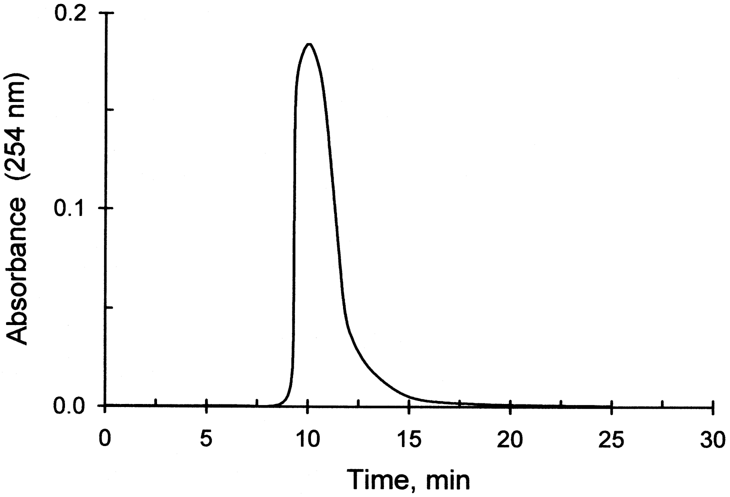

Figure 1. CCC extraction of pyrene and other components of the sewage sludge. Stationary phase (sample): sewage sludge and its mixture with aqueous solution of pyrene. Mobile phase: n-heptane-dichloromethane (4:1). F = 1 mL/min. UV-detection at 254 nm.

data obtained (Table 2) we can assume that the recovery of pyrene is quantitative. As is seen from Table 2, the sewage sludge under investigation contains pyrene at the level of 0.24 μg/mL. This value is in good agreement with possible contents of pyrene in the sewage sludge samples.[6]

The corresponding HPLC chromatograms of the effluent obtained using fluorescence detection are presented in Figure 2. Using chromatogram 1 we can identify the peak of pyrene on other chromatograms. As is seen, chromatograms 2 and 3 are similar, except the peak of pyrene. The second peak appearing on these chromatograms may correspond to another PAH. If a larger volume (4 mL) of the sewage sludge is analyzed (chromatogram 4), some other PAHs are detected.

Separation of Naphthalene, Pyrene, and Benzo(g,h,i)perylene

The solubility of PAHs in water is low, especially for benzo(g,h,i)perylene (less than 0.1 μg/mL).[16] However, this quantity is sufficient for the detection of PAHs by UV absorptiometry.

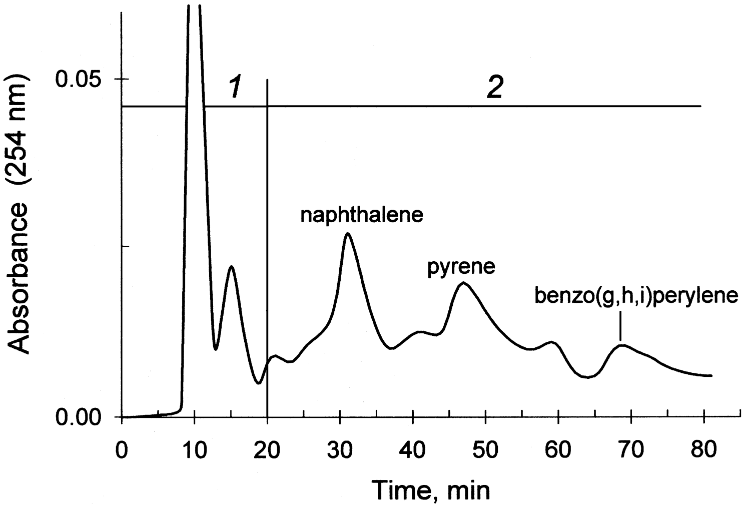

Figure 2. HPLC analysis of the CCC column effluent. Fluorescence detection at 295/380 nm. Samples: see Table 2.

Using a saturated aqueous solution of naphthalene, pyrene, and benzo(g,h,i)perylene as the stationary phase in the column, we have found the mobile phase and experimental conditions for the separations of these compounds to be achieved.

If the concentration of dichloromethane in the mobile phase progressively increases form 0 to 20%, a subsequent elution of PAHs from the column becomes possible. Hence, PAHs can be separated by the use of a gradient elution mode.

The separation of naphthalene, pyrene, and benzo(g,h,i)perylene from the sewage sludge medium is shown in Figure 3. At the first stage, constituents of the sewage sludge well soluble in n-heptane are eluted, whereas PAHs remain in the stationary phase. Then, with increasing the dichloromethane concentration in the mobile phase, the peaks of naphthalene, pyrene, and benzo(g,h,i) perylene appear. On the chromatogram one can see other peaks which may correspond to other PAHs and contaminants present in the sewage sludge.

CONCLUSIONS

Using CCC, the analysis of complex natural matrices without sample pretreatment is principally possible. The stationary phase (sample) containing sus-

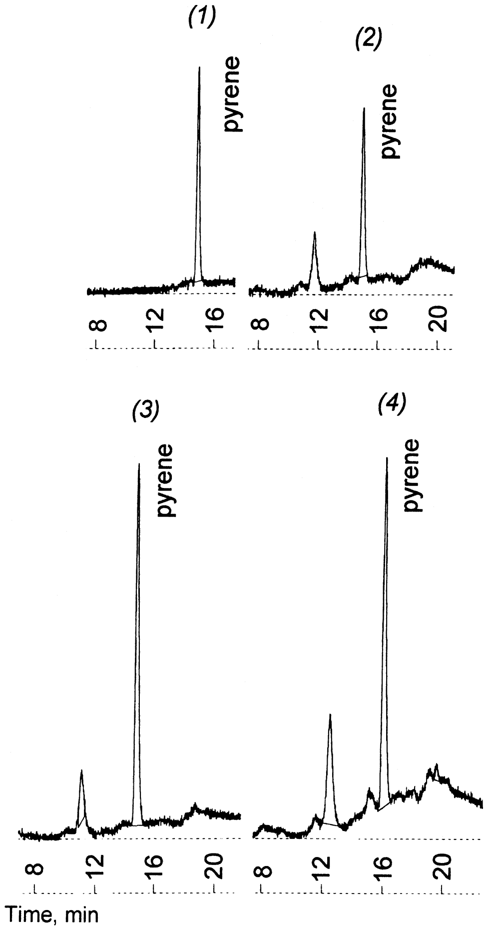

Figure 3. Separation of naphthalene, pyrene, and benzo(g,h,i)perylene from the sewage sludge medium. Stationary phase (sample): saturated aqueous solution of enumerated PAHs, 2 mL + sewage sludge, 2 mL. Mobile phase: *1* - n-heptane; *2* - n-heptane-dichloromethane; gradient elution mode (concentration of dichloromethane increases from 0 to 20%). *F* = 1 mL/min. UV-detection at 254 nm.

pended solid particles can be successfully retained in the CCC column. Although the subsequent separation of naphthalene, pyrene, and benzo(g,h,i)perylene from the sewage sludge medium has been demonstrated, the group separation of PAHs followed by their quantitative determination by another instrumental technique may be more profitable.

To our opinion, the present work is the first example of application of CCC in the field of ecology and environmental chemistry. Further studies are needed to extend the results obtained to other PAHs and contaminants in various complex samples.

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