

# Aryl Chemically Bonded Phases for Determination of Selected Polycyclic Aromatic Hydrocarbons Isolated from Environmental Samples Utilizing SPE/HPLC

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Received 21 June, 1999

Accepted 1 July, 1999

## Abstract

A method for the preparation and application of aryl chemically bonded phases designed for SPE/HPLC determination of polycyclic aromatic hydrocarbons (PAHs) in environmental samples is described. New adsorbents have been characterized by different physicochemical techniques (porosimetry, CHN and chromatographic analysis). The sorption properties of prepared packings were investigated by the determination of sorption isotherms (so-called breakthrough curves). A comparison of the recovery rates (R) of selected PAHs for new packings and for conventional material C<sub>8</sub> has been performed.

**Keywords:** aryl chemically bonded phases, solid phase extraction (SPE), optimization of the isolation process, recovery rate

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute one of the most investigated groups of carcinogenic compounds dangerous for human health. Their concentration in the natural environment has been increased in the latest years from a few dozen to several hundred times [1-4]. The presence of PAHs in all elements of the environment (water, soil, air, biota) has caused health hazards connected with their activity to have a general character. Because of low concentrations of analytes (ppb, ppt level), the first step - a proper preparation of samples before the essential determination (e.g. spectrophotometric or chromatographic) is very important and has an influence on the final

results. The sample preparation consists in an isolation of microcomponents from a matrix and later enrichment, until the concentration sufficient for determination (UV-Vis, fluorescent or diode array detection) is obtained [1, 4-6]. Among methods of enrichment and isolation of micro-traces the most important are liquid-liquid extraction (LLE), gas-solid extraction (GSE) and solid-phase extraction (SPE) [7,8].

Recently, solid-phase extraction has been distinguished as the most universal method used in *on-line* and *off-line* systems [7-9]. A wide progress of SPE has been achieved due to the introduction of numerous new types of packings. These materials consist mainly of chemically bonded phases, prepared on the base of silica gel [10-13]. For the separation of PAHs (containing electrons) aryl [14, 15], phenyl (with arcade-like structure) [16, 17] and fluorenyl

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phases [18, 19], in which  $\pi$ -interactions are dominating, are proposed. These phases were precursors of packings with expanded geometry, i.e. fullerene packings used for the separation of hydrocarbons ( $C_{60}$ ,  $C_{70}$ ,  $C_{76}$ ,  $C_{80}$ ) [15]. Considering these specific interactions, one can understand that preparation of phases for the separation of this type of compound is difficult and only a few papers concerning this problem have been published [20].

In this paper, the preparation of packings with aryl chemically bonded phases (containing one or more benzene rings) is described. The packings were successfully applied to enrichment and isolation of polycyclic aromatic hydrocarbons from environmental samples (soil, dandelion, ribwort) by a combined technique SPE/HPLC. New adsorbents were characterized using different physicochemical methods (i.e. porosimetry, ICP,  $^{13}C$  CP MAS NMR) and their sorption properties were investigated by determination of so-called breakthrough curves. On the basis of investigation results, a column has been chosen for enrichment and isolation of PAH from soil, leaves of dandelion (*Taraxacum officinale* W) and ribwort (*Plantago major*) after extraction in a Soxhlet apparatus. Qualitative and quantitative determination was performed by HPLC with UV-Vis detection.

## Experimental

### Reagents and Materials

Packings with chemically bonded phases were prepared on the base of Kieselgel Si-60 irregular silica gel (Merck,

Table 1. Physical-chemical characteristics of silica gel Kieselgel Si-60 used as a support of chemically bonded phases.

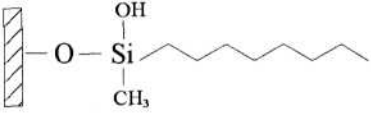
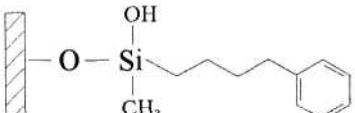
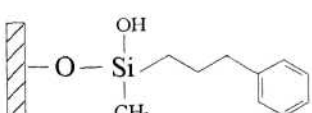
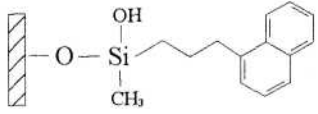
Parameter	Abbreviation	Unit	Value
Particle shape	KZ	-	irregular
Particle size	$d_p$	$\mu m$	40-63
Porosity:			
Specific Surface area	$S_{BET}$	$m^2/g$	320
Pore diameter	D	nm	5.8
Pore volume	$V_p$	$cm^3/g$	0.884

Darmstadt, Germany). Physicochemical characteristics of the adsorbent are presented in Table 1. The gel surface was modified by use of dichlorodimethylsilane (POCh, Gliwice, Poland). Specially dried morpholine (Reachim, Moscow, Russia) served as an activator. Reagents: hexane, methanol, toluene, tetrahydrofuran (THF), acetonitrile, 2-propanol, petroleum ether, methylene chloride were *HPLC-grade* (S. Witko - J.T. Baker, Lodz, Poland). As standards a mixture of benzene, naphthalene, biphenyl, anthracene, pyrene, 1,2-benzoanthracene, chrysene (Fluka AG, Buchs, Switzerland) and single test substance - chrysene were used. All standards were dissolved in acetonitrile *HPLC-grade*.

For the optimization of the extraction process in a liquid-solid system, a series of columns was used, consisting of octyl (Si-C<sub>8</sub>), phenylbutyl (Si-FB), phenylpropyl (Si-AB) and naphthylpropyl (Si-NAF). Characteristics of the packings used for SPE are presented in Table 2.

Table 2. Characteristics of prepared packings with chemically bonded phase

Where:  $\alpha_{RP}$  - concentration of bonded alkyl or aryl ligands,  $P_c$  - percentage of carbon,  $P_H$  - percentage of hydrogen.

No of column	Type of CPBs structure	Structure	Phase sode	Coverage denisty		$\alpha_{RP}$ [ $\mu mol/m^2$ ]
				$P_c$ [%]	$P_H$ [%]	
1.	Octyl		Si-C <sub>8</sub>	13.49	3.08	4.78
2.	Phenylbutyl		Si-FB	14.94	2.59	5.11
3.	Phenylpropyl		Si-AB	13.32	2.83	4.93
4.	Nafthyl-propyl		Si-NAF	16.1	2.23	4.51

## Apparatus

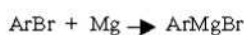
Solid-phase extraction was performed in a pressure chamber, type SPE 12G, made of borosilicate glass (J.T. Baker, Gross Gerau, Germany). Isolation of PAH from soil, dandelion and ribwort was carried out in a Soxhlet extractor (volume 50 cm<sup>3</sup>) with 200 cm<sup>3</sup> flask (Altech Association, Carnforth, England). The extracts were concentrated using a rotary evaporator (type RE 100, Bibby Sterlin LTD, Stone Staffordshire, England).

Chromatography was performed with an HPLC SPD-6A (Shimadzu, Kyoto, Japan) system consisting of a model LC-6A pump, a Rheodyne model 7125 injector with a 20 µl sample-loop, UV detector and a model C-R6A recorder. A stainless steel column (125 x 4.6 mm I.D.) filled with Si-NAF (Berkeley, CA, USA) was used.

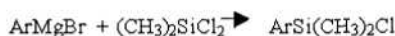
### Preparation of Aryl Packings and SPE Columns

The chemical modification of silica gel surface Kieselgel Si 60 was carried out according to the static method described in [22], which enables obtaining packings with so-called *thick* and *controlled* covering. Packings obtained by this method were washed on a Schott funnel with toluene, methanol and hexane in turn. Then, the packings were dried at room temperature (20°C ± 1). Packings with aryl groups (Si-FB, Si-AB and Si-NAF) were prepared according to the three-stage reaction scheme presented in Fig. 1.

#### I stage



#### II stage



#### III stage

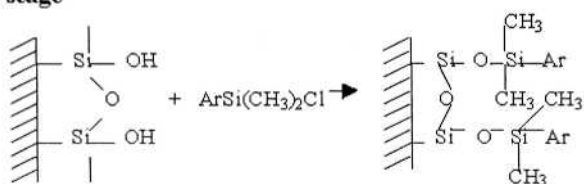


Fig. 1. Scheme of three-stage reaction of obtaining of aryl phases.

Samples of new stationary phase (1.4 g weighed on an analytic balance) were quantitatively transferred to an extraction column (polyethylene trunks of disposable syringes), produced by ZPF-Polfa (Lublin, Poland). Adsorbent was homogeneously placed and protected with polyurethane discs with diameter of mesh 15-20 µm (Alltech Association, Carnforth, England), which prevented the migration of adsorbent particles during the elution process.

### Methodology of Measurements

Samples of soil, dandelion and ribwort were collected in Siedlce (Brzeska St), in summer, in the neighbourhood of

a petrol station. This place was chosen due to increased accumulation of PAHs. Samples were analyzed according to the scheme (Fig. 2).

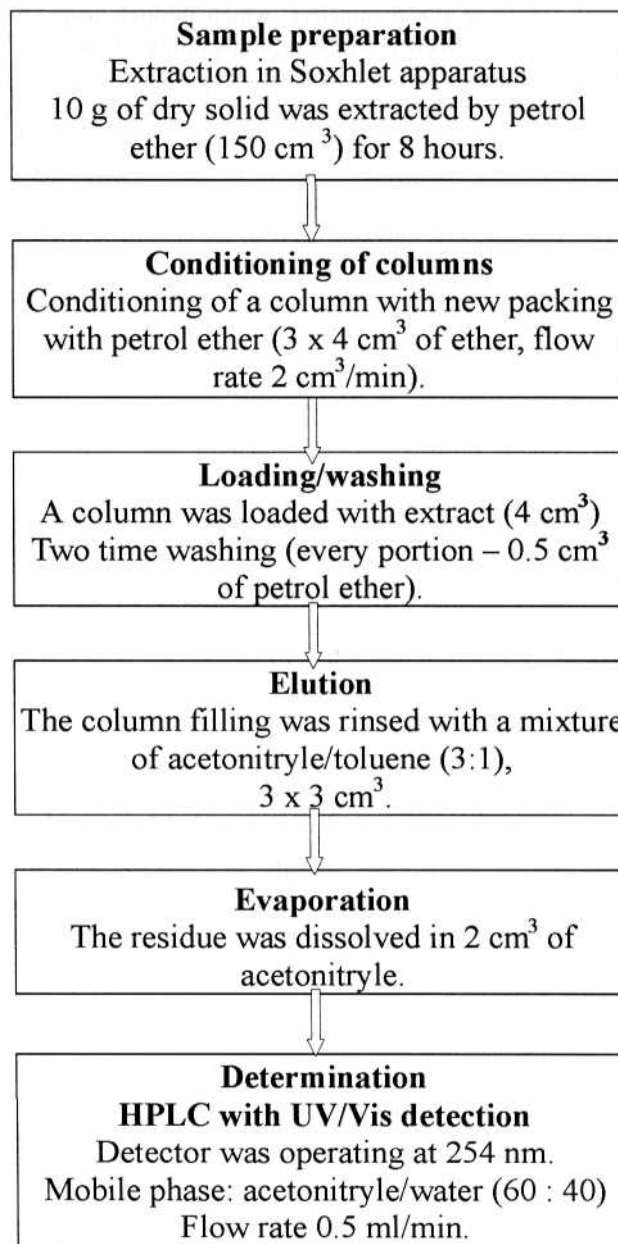


Fig. 2. Scheme of the preparation and analysis of samples by SPE/HPLC.

## Results and Discussion

### Characteristics of Packings and Surface Topography

Data describing properties of new adsorbents with chemically-bonded phases are shown in Table 2. All packings were prepared on the base of silica gel Kieselgel Si-60 (characteristics of the Kieselgel surface are presented in Table 1.) Porosity data of new packings (specific surface, pore diameter, pore volume) fulfil requirements for SPE adsorbents [8, 9]. Surface porosity, represented by the specific surface ( $S_{\text{BET}}$ ) and pore diameter ( $D$ ), is decisive for

mass exchange; therefore, porosity determines recovery rate values ( $R$ ). On the other hand, homogeneity and thickness of the undersurface structure of chemically bonded phase constitute the parameters, which through recovery rates have an influence on the reproducibility of the sorption of analytes, which are isolated and enriched from matrices. Values of carbon percentage for all investigated adsorbents are situated between 13.2-16.1% (Table 2). It shows that the effectiveness of blocking of undersurface silanol groups during the formation of chemically bonded phase is good. The values of carbon percentage are comparable with data published by Tanaka [13, 14] and Albert [22, 23], although systems described there have more expanded structure of bonded ligands.

It can be seen from the values of chrysene recovery rates (Fig. 3), that the highest value ( $R \approx 75 \pm 5\%$ ) was obtained for columns with Si-NAF packing. Most probably, this is due to specific selective sorption, with dominating participation of  $\pi$ - $\pi$  type interactions, between chosen molecules of an analyte (chrysene), which contains high energy  $\pi$  electrons, with  $\pi$  electrons of naphthalene (attached to the silica gel surface by a short alkyl chain). In remaining cases only slight differences of  $R$  values can be observed (Fig. 3), i.e. at about  $70 \pm 2\%$ . High values of recovery rates and a good reproducibility of the process (low values of S.D., under 5%) are ensured by the presence of expanded molecules, which are attached by alkyl chains to the silica gel surface in order to block access of an analyte to the residual silanol groups. This effect was investigated in detail by Jinno [20] and Albert [23]. On the other hand, elongation of the alkyl chain by one carbon atom (Table 2; phase #2 and #3) has an insignificant (in limits of error, S.D.  $\pm 2\%$ ) influence on recovery rate values. However, it can be a result of increased mobility of bonded ligands.

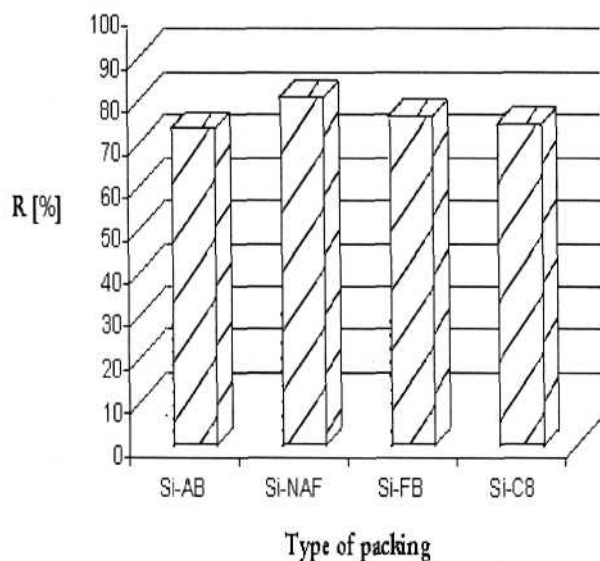


Fig. 3. A comparison of recovery rate values ( $R$ ) for chrysene, obtained on different packings with aryl chemically bonded phases (symbols of columns as in Table 2).

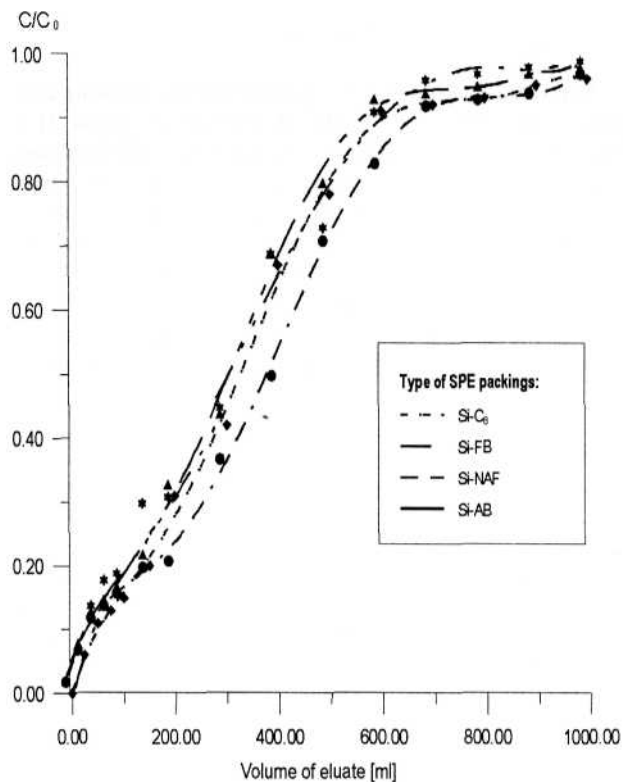


Fig. 4. Column breakthrough curves for new packings for SPE on the example of chrysene at a concentration of  $10^{-5}$  ppm.

An effect of various interaction of an analyte with packings is confirmed by column breakthrough curves obtained for new adsorbents (Fig. 4). The shape of the curve, obtained for octyl phase, where two kinds of active centres are present on the silica gel surface (residual silanol groups and hydrophobic alkyl chains) are practically close to the theoretic one (*ideal*) [8, 9]. The same situation can be observed in cases of adsorbents Si-FB and Si-AB, where as terminal groups serve molecules of benzene differing by the length of alkyl chain. If a terminal group with increased number of  $\pi$  electrons is situated on the surface (more complicated structure, e.g. Si-NAF), the shape of column breakthrough curve only slightly differs from the ideal. It can suggest participation of  $\pi$  electrons in the sorption process; however, values of  $c/c_0$  are not significantly changed.

#### Application

A column filled with Si-NAF was applied to the isolation and enrichment of PAH from samples of soil, leaves of dandelion and ribwort. Adsorbent has been chosen because of high and repeatable recovery rate ( $R$ ) and good reproducibility (S.D. =  $\pm 4.1\%$ ) for the test compound - chrysene. Extracts of environmental samples were prepared according to the procedure shown in Fig. 2. On the basis of chromatographic analysis benzene, biphenyl and five PAHs (naphthalene, anthracene, pyrene, 1,2-benzoanthracene, chrysene) were identified. Their concentrations, depending on the matrix, were from 0.5 to  $10^{-4}$  ppm (Table 3).

Table 3. Determination of benzene, selected PAHs and biphenyl in different environmental matrices by SPE/HPLC on aryl phase Si-NAF (SD <math>\pm 5\%</math>).

Compound	soil [ppm]	ribwort [ppm]	dandelion [ppm]
Benzene	0.5242	$1.859 \cdot 10^{-2}$	$9.45 \cdot 10^{-4}$
Naphthalene	0.5498	0.1436	$5.627 \cdot 10^{-2}$
Biphenyl	$4.885 \cdot 10^{-2}$	$6.514 \cdot 10^{-2}$	$2.32 \cdot 10^{-4}$
Anthracene	0.2067	$4.064 \cdot 10^{-2}$	$5.614 \cdot 10^{-3}$
Pyrene	$8.454 \cdot 10^{-2}$	2.549	$8.717 \cdot 10^{-3}$
1,2-benzanthracene	$3.721 \cdot 10^{-3}$	$2.58 \cdot 10^{-4}$	$2.257 \cdot 10^{-5}$
chrysene	$6.483 \cdot 10^{-4}$	nd	nd

nd – not detected

### Conclusion

As a result of extraction, optimization on columns with aryl phases in a liquid-solid system was stated as follows:

1. Preparation of aryl phases enables obtaining of reproducible packings with aryl phases with thickness of covering at about  $5 \mu\text{mol}/\text{m}^2$ . It confirms effective blocking of superficially accessible hydroxyl groups situated on the silica gel surface.

2. An influence of length of the chain attached to the terminal phenyl group is manifesting itself in considerable (at about 20%) differences in values of sorption capacities in a range from 0 to 50 ml. On the other hand, the participation of naphthyl group as a terminal element of phase Si-NAF does not significantly change the  $c/c_0$  values. It may be attributed by the creation of a different undersurface structure of the phase {conformation change}.

3. An effect of shielding of the surface is visible in high and reproducible values of recovery rate(R) of isolated and enriched of PAHs, obtained using Si-NAH phase.

4. A column with Si-NAF packing enables obtaining of reproducible values of R due to selective sorption as a consequence of interactions - type between the stationary phase and an investigated analyte (chosen PAH). Measuring errors do not exceed 5% in all cases.

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