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# Characterisation of the chromatographic properties of a silica–polypyrrole composite stationary phase by inverse liquid chromatography

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## Abstract

Silica–polypyrrole particles have been used as a composite stationary phase for liquid chromatography. Determination of capacity factors ( $k'$ ) of a wide number of polycyclic aromatic hydrocarbon (PAH) molecular probes allows the characterisation of the chromatographic properties of the silica–polypyrrole stationary phase. Capacity factors in the range of 0.10 up to 6.1 were determined, thus demonstrating the high affinity of the PAH probes towards the stationary phase. The selectivity of the composite stationary phase was also evaluated as a function of the planarity of the molecular probes injected. Capacity factors determined for PAHs (two-dimensional molecular probes) are higher than those measured for phenyl-substituted PAHs (phenyl-PAHs, three-dimensional molecular probes). Determination of capacity factors, dependence on the composition of the mobile phase demonstrates the reverse  $\alpha$ -phase properties of the composite stationary phase. The acid–base properties of the composite silica–polypyrrole stationary phase were investigated using benzene derivative molecular probes (i.e., toluene, phenol, benzoic acid and aniline). Capacity factors in the range of 0.45 to 1.0 were determined. This study clearly demonstrates that this composite stationary phase exhibits selective interactions towards PAHs and phenyl-substituted PAHs and strong acid–base properties depending on the structure, the geometry and the acid–base properties of the molecular probes eluted.

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## 1. Introduction

Improvement of the chromatographic properties of a silica stationary phase can be achieved by chemical modification of the surface properties of the silica

particles. Silane-modified and polymer-grafted silica particles have attracted a considerable attention for the development of new stationary phases for liquid chromatography (LC). Non-polar stationary phases can be obtained by grafting alkylsilane onto silica [1,2]. Highly polar stationary phases can be prepared by reacting functionalised silane [1–3] (silane bearing amino, cyano, carboxylic or phenyl pendant groups) or by grafting polymeric materials [4] onto the silica surface. Studies of chromatographic be-

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haviour of polycyclic aromatic hydrocarbons (PAHs) and methyl- or phenyl-substituted PAHs (Me-PAHs and phenyl-PAHs, respectively) by high-performance liquid chromatography (HPLC), have been widely reported to characterise the separation selectivity and performance for various stationary phases and mobile phase compositions [5]. Chromatographic retention is also used to establish correlation dependencies between chromatographic parameters and various physico-chemical properties and/or molecular structure of PAH molecules. This mathematical modelling enables the prediction of the properties of chromatographic systems and to optimise mobile phase composition and conditions for separation. Physico-chemical properties of PAH molecules used to correlate chromatographic retention are mainly the number of aromatic cycles and carbon atoms in the PAH molecules [6,7], Van der Waals volume [8], length-to-breadth ratio ( $L/B$ ) [9,10], hydrophobic character ( $\log P$ ) [11,12], the molecular connectivity index ( $\chi$ ) [13],  $\pi$ -electron energies [14], in addition to the molecular structure-behaviour relationship [15,16].

Conducting polymers, such as polypyrrole (PPy), polyaniline (PANI) and poly(3,4-ethylenedioxythiophene) (PEDOT), constitute a special class of polymeric materials due to their interesting surface properties. These polymers exhibit high dispersive contribution to the total surface free energy ( $\gamma_s^d$ ), present an amphoteric character and can also be used in ion-exchange processes [17]. These interesting surface properties suggest promising use of these polymers as new polymeric materials for stationary phases in chromatography. Chehimi and co-workers used polypyrrole powders, doped with various anion dopant, as a stationary phase for inverse gas chromatography (IGC) [18,19]. These studies demonstrated that polypyrrole exhibits a high dispersive contribution to the total surface free energy ( $\gamma_s^d$ ) and is an amphoteric material with a predominant acidic character. Chehimi and co-workers also characterised composite silica-PPy particles by IGC and showed that these particles exhibit a stronger dispersive contribution to the total surface energy ( $\gamma_s^d$ ) and acid-base properties compared to polypyrrole powders [20,21]. Wallace and co-workers grafted conducting polypyrrole and PANI onto silica particles. The chromatographic properties of composites sil-

ica-PPy and silica-PANI stationary phases were characterised using a series of PAHs and small drugs molecular probes by LC [22–25]. They demonstrated that polypyrrole exhibits higher selectivity and affinity towards the molecular probes compared to polyaniline. However, the low surface content of conducting polymer of these composite particles suggests that both the silica substrate and conducting polymer overlayer govern the retention mechanism of the probes.

In previous work, we reported the use of silica particles as high surface area substrate for the chemical synthesis of conducting polypyrrole overlayer (PPy). This study demonstrated the influence of the pre-treatment of the silica particles with a specific organosilane (aminopropylsilane, APS) prior polypyrrole coating. The synthesis and characterisation of both bulk and surface properties of new conducting composite silica-PPy and silica-APS-PPy particles have been previously reported [24,25]. The composite silica-PPy particles exhibit a silica-rich surface, and thus, the surface properties are dominated by the silica substrate. In contrast, the composite silica-APS-PPy particles present a high polypyrrole surface content, and thus, the surface properties are governed by the intrinsic polypyrrole properties. Preliminary attempts to use the composite silica-APS-PPy particles as stationary phase for IGC were unsuccessful as conducting polypyrrole material is a high surface energetically materials as demonstrated by Chehimi et al. for polypyrrole powders [21]. Due to the high surface area of the silica-APS-PPy particles ( $180 \text{ m}^2 \text{ g}^{-1}$ ), molecular probes interact strongly and/or adsorb irreversibly on the composite surface and could not be eluted. LC was thus chosen as alternative technique to characterise the chromatographic properties of silica-APS-PPy as novel composite stationary phase.

In this work, the determination of capacity factors ( $k'$ ) of series of PAHs and phenyl-PAHs allow the characterisation of the affinity and the selectivity of the silica-APS-PPy stationary phase towards these molecular probes. Attempts are made to correlate the capacity factors of PAH probes to physico-chemical properties and to a molecular structure descriptor. Elution of molecular probes, depending the composition of the mobile  $\alpha$ -phase, demonstrates the reversed-phase character of the silica-APS-PPy

stationary phase. The acid–base properties of the silica–APS–PPy stationary phase were also investigated by using a series of benzene derivative probes bearing neutral (i.e., toluene), acidic (i.e., benzoic acid and phenol) or basic (aniline) functional groups, respectively. The values obtained are compared to a commercial octadecyl-modified silica stationary phase (i.e., silica–C<sub>18</sub>).

## 2. Experimental

### 2.1. Composite silica–polypyrrole stationary phase

The full details concerning the synthesis and characterisation (bulk and surface physico–chemical properties) of the composite silica–polypyrrole particles have been previously published [26–28]. This previous work clearly demonstrated that pre-treatment of the silica gel particles by a specific silane-coupling agent (i.e., APS), prior to polypyrrole coating, allows a relative increase the surface content of the polypyrrole overlayer compared to untreated silica gel particles. The synthesis of the composite particles was obtained in two steps. First, the silica particles (Merck, Paris, France; particle diameter in the range 60–125  $\mu\text{m}$ , porous volume = 0.75 ml g<sup>-1</sup>) were pre-treated with 1% (v/v) APS (Acros, Loughborough, UK; 99%) in ethanol–water. Then, pyrrole monomer (Acros, 99%) was adsorbed onto the silica–APS particles, followed by oxidative chemical polymerisation in aqueous solution. In this study, silica–APS–PPyTS (TS: tosylate dopant species) particles were chosen due to the high polypyrrole surface content, compared to silica–PPyTS (silica-rich surface). The specific surface area of the silica–APS–PPyTS stationary phase was 180 m<sup>2</sup> g<sup>-1</sup> as determined by BET measurements.

### 2.2. Columns

A commercial octadecylsilane-modified silica (i.e., silica–C<sub>18</sub>), supplied by Chrompack (Paris, France), of 15×0.4 cm, silica particle size of 10  $\mu\text{m}$  and pore size of 80 Å, was used as a reference column. The silica–APS–PPyTS particles were suspended in acetonitrile and then packed into a column (15×0.4 cm) at 280 bar.

### 2.3. Reagents and solutes

A mixture of acetonitrile and Milli-Q water (Prolabo, Paris, France; HPLC grade) was used as mobile phase for all experiments. The volume fraction of acetonitrile–water in the mobile phase was fixed at 90:10 (v/v) in all experiments, unless otherwise stated.

Benzene (Prolabo, normapur), and the PAHs naphthalene (Acros, 99%), phenanthrene (Acros, 99%), anthracene (Acros, 99%), pyrene (Aldrich, St. Quentin Fallavier, France; 99%), chrysene (Acros, 99%), 1,2-benzanthracene (Acros, 99%), 2,3-benzanthracene (Aldrich, 98%), perylene (Acros, 99%) and triphenylene (Acros, 98%); phenyl-PAHs: biphenyl (Acros, 99%), 1-phenylnaphthalene (Aldrich, 96%), *o*-terphenyl (Aldrich, 99%), *m*-terphenyl (Aldrich, 99%), *p*-terphenyl (Aldrich, 99%), 9,10-diphenylanthracene (Acros, 97%) and 1,3,5-triphenylbenzene (Acros, 97%); and benzene derivatives: toluene (Prolabo, 99.5%), phenol (Acros, 99%), benzoic acid (Acros, 99%) and aniline (Acros, 99%), were used as received. The molecular structures of the PAHs and phenyl-PAHs used in this work are presented in Fig. 1.

Solutions of 15 and 30 ppm of PAHs, phenyl-PAHs and benzene derivative molecular probes, were prepared in pure acetonitrile. It was checked that the retention time (or capacity factor *k'*) was independent of the concentration of the molecular probe injected, and thus, that not all interacting sites at the surface of the stationary phase were saturated at these concentrations.

### 2.4. Instrumentation

Determination of the chromatographic properties was performed using a HPLC system based on a Kontron 325 pump (Palaiseau, France), which allows the control of the flux and composition of the acetonitrile–water mobile phase mixture; a Rheodyne 7161 injector with a 20  $\mu\text{l}$  sample loop; and a Dionex AD20 UV–visible variable-wavelength detector (St. Quentin en Yvelines, France).

### 2.5. Chromatographic measurements

The flow of the mobile phase was set to 0.5 ml

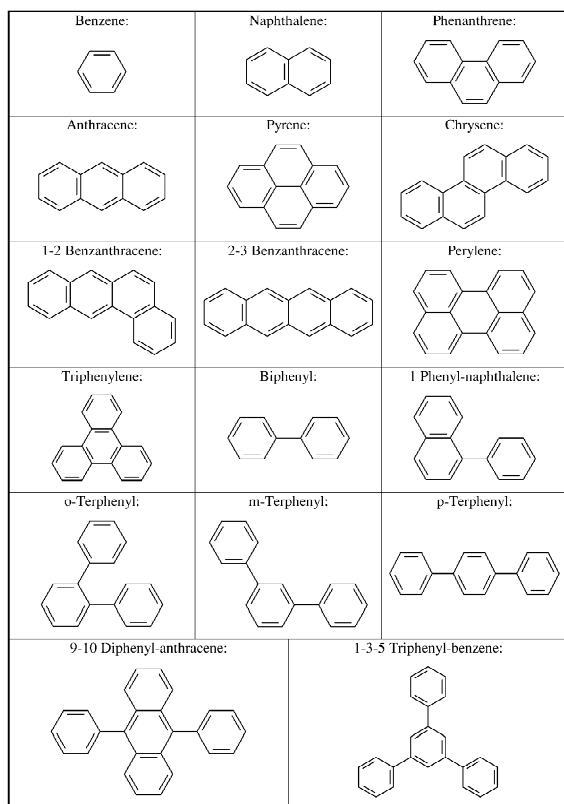


Fig. 1. Molecular structure of PAHs and phenyl-PAHs used in this work.

$\text{min}^{-1}$  during analysis. A mixture of acetonitrile–water (90:10, v/v) was used as mobile phase for all experiments, unless otherwise stated. The solute probe output signal was monitored at a wavelength of 254 nm. Chromatograms were recorded and retention times were determined using Borwin JMBS software. Before analysis, each column was flushed overnight with an acetonitrile–water solution mixture at a given volume proportion.

## 2.6. Molecular descriptors

Several molecular descriptors have been used to correlate the behaviour of the molecular probes to the capacity factors as measured by HPLC.

### 2.6.1. Van der Waals volume ( $V_{vdw}$ )

The Van der Waals volume of the PAH molecules is determined from the Van der Waals radii of the

carbon and hydrogen atoms involved in the molecule [29].

### 2.6.2. Connectivity index ( $\chi$ )

The connectivity indexes ( $\chi$ ) were calculated according to the method of Kaliszan and Lamparaczyk for the PAHs [13]. The connectivity index parameter describes the topological size and the degree of branching of the molecule.

### 2.6.3. Partition coefficient

The logarithm of the partition coefficient ( $\log P$ ) measures the solubility of the PAH molecules in a 1-octanol–water mixture [30]. This parameter determines the hydrophobic properties of the molecule.

### 2.6.4. Correlation factor ( $F$ )

Schabron et al. introduced the correlation factor ( $F$ ) to describe chromatographic retention of PAH molecules in liquid chromatography [31]. The correlation factor ( $F$ ) is calculated as follows:

$$F = (\text{number of double bonds}) + (\text{number of primary and secondary carbon atoms}) - 0.5 \times (\text{number of nonaromatic rings})$$

### 2.6.5. Polarisability ( $\alpha$ )

Due to their aromatic structure, the electron cloud of the PAH molecules can be easily polarized by neighbour molecules. The polarisability ( $\alpha$ ) is defined according the strength of the induced dipole moment  $\mu_{\text{ind}}$  acquired in a field  $E$  ( $\mu_{\text{ind}} = \alpha E$ ) [32].

### 2.6.6. Length ( $L$ ) and length-to-breadth ratios ( $L/B$ )

The length ( $L$ ) and length-to-breadth ratio ( $L/B$ ) for a series of PAHs were calculated using the data of Radecki et al. [33]. The length-to-breadth ( $L/B$ ) represents the ratio of the maximised length-to-breadth of a rectangle box enclosing the PAH molecule. Since PAH molecules are coplanar, the  $L/B$  ratio can be used as a two-dimensional geometrical descriptor of the shape of the molecule.

Table 1  
Physico-chemical parameters of PAH molecular probes

Molecular probe	Molecular mass (g mol <sup>-1</sup> )	Van der Waals volume (ml mol <sup>-1</sup> )	Molecular connectivity index ( $\chi$ )	Partition coefficient (log $P$ )	Correlation factor ( $F$ )	Polarisability ( $\alpha$ )	Length ( $L$ )	Length-to-breadth ratio ( $L/B$ )
Benzene	78.11	48.36	2.000	2.16	3.0	9.03	7.41	1.10
Naphthalene	128.17	73.96	3.405	3.18	5.0	17.48	9.20	1.24
Phenanthrene	178.23	99.56	4.815	4.20	7.0	24.70	11.75	1.46
Anthracene	178.23	99.56	4.809	4.20	7.0	25.93	11.65	1.57
Pyrene	202.26	109.04	5.559	4.50	8.0	29.34	11.66	1.27
Chrysene	228.29	125.16	6.226	5.22	9.0	33.06	13.94	1.72
1,2-Benzanthracene	228.29	125.16	6.220	5.22	9.0	32.86	13.94	1.58
2,3-Benzanthracene	228.29	125.16	6.214	5.22	9.0	34.38	14.11	1.89
Perylene	252.32	134.64	6.976	5.52	10.0	37.77	11.80	1.27
Triphenylene	228.29	125.16	6.232	5.22	9.0	33.50	11.68	1.12

### 3. Results and discussion

#### 3.1. PAH molecular probes

Fig. 1 reports the molecular structures of the PAH molecular probes used in this work. Table 1 reports the physico-chemical properties and the values of several molecular descriptors used to characterised polyaromatic hydrocarbon molecules.

The capacity factors measured for silica-APS-PPyTS and commercial silica-C<sub>18</sub> stationary phases for a series of PAH molecular probes, with a mobile phase of acetonitrile-water (90:10, v/v), are presented in Table 2. For comparison, Table 2 also contains capacity factors reported by Chriswanto et al. for a silica-PPyDS (DS: dodecylsulfate dopant species) stationary phase, and using a similar mobile phase composition [22].

Table 2 clearly demonstrates that the capacity factors of PAH molecules obtained for the silica-APS-PPyTS stationary phase are equal or higher than those previously reported by Chriswanto et al. for a silica-PPyDS stationary phase in similar conditions [22]. This result can be explained by both the high polypyrrole surface content of the composite silica-APS-PPyTS particles, as previously demonstrated by X-ray photoelectron spectrometry (XPS) and secondary ion mass spectrometry (SIMS) analyses [26–28], and also by the nature of the dopant tosylate (TS) species used in the conducting polymer. Chehimi and co-workers reported a dispersive contribution to the total surface energy ( $\gamma_s^d$ ) for polypyrrole powders in the range of 80–150 mJ m<sup>-2</sup> at 60 °C, as measured by IGC [18–21]. Wallace and co-workers demonstrated that the nature of the dopant species incorporated in polypyrrole also

Table 2  
Capacity factors ( $k'$ ) of PAH molecular probes determined for silica-C<sub>18</sub>, silica-APS-PPyTS and silica-PPyDS [22] stationary phases with acetonitrile-water (90:10, v/v) as mobile phase

Molecular probe	Silica-C <sub>18</sub> (Chrompack)	Silica-APS-PPyTS	Silica-PPyDS [22]
Benzene	0.30	0.03	–
Naphthalene	0.51	0.10	0.04
Phenanthrene	1.04	0.96	0.63
Anthracene	1.31	1.17	0.77
Pyrene	1.94	2.20	2.52
Chrysene	3.49	6.09	4.93
1,2-Benzanthracene	3.04	5.68	5.69
2,3-Benzanthracene	2.51	≥ 6	–
Perylene	5.50	≥ 6	28.30
Triphenylene	2.16	4.83	4.82

influence the chromatographic properties of a composite silica–polypyrrole stationary phase by liquid chromatography [22,25,34]. It was clearly demonstrated that dodecylsulfate dopant species interacts stronger with basic drug, small proteins and polyaromatic hydrocarbons than chloride dopant species. These results were interpreted in terms of higher hydrophobicity, and thus stronger interactions of the molecular probes with the stationary phase, induced by the dodecylsulfate dopant species incorporated in the polypyrrole backbone. Recent results from our laboratory demonstrated that the contact angle formed by a drop of water on electrochemically-synthesized polypyrrole films depends on the nature of the dopant anion species incorporated in the film [35]. This result demonstrates that the nature of the dopant greatly influences the degree of hydrophobicity of the surface of the polypyrrole film. Tosylate anion dopant induced the highest contact angle. For this reason, tosylate was chosen as anion dopant species to be incorporated in the polypyrrole matrix.

In order to understand the behaviour of the PAHs towards silica–APS–PPyTS and silica–C<sub>18</sub> stationary phases, the capacity factors measured for the PAH molecular probes are correlated to various inherent physico–chemical properties or molecular descriptors.

### 3.2. Capacity factors and PAH molecular masses

Capacity factors of the PAHs increase by increasing molecular mass of the molecular probes (see Tables 1 and 2). For low-molecular-mass PAH molecular probes (i.e., benzene, naphthalene, phenanthrene, anthracene), higher capacity factors are observed for silica–C<sub>18</sub> compared to silica–APS–PPyTS. However, for high-molecular-mass PAH molecular probes (i.e., pyrene, chrysene, 1,2-benzanthracene, triphenylene), higher capacity factors are observed for silica–APS–PPyTS compared to silica–C<sub>18</sub>. This result demonstrates that the nature of the stationary phase strongly influences the interaction occurring between the surface of the stationary phase and the molecular probe. Silica–APS–PPyTS stationary phase interact strongly with PAHs of high molecular mass.

However, for the series phenanthrene/anthracene

and chrysene/1,2-benzanthracene/triphenylene, even though these molecular probes have similar molecular masses, different capacity factors are observed. This result demonstrates that the molecular mass alone cannot be used to predict the order of elution of molecular probes.

In a similar manner, increasing capacity factors of the PAHs are observed as the Van der Waals volume ( $V_{vdw}$ ), partition coefficient ( $\log P$ ) and correlation factor ( $F$ ) of the molecular probes increase (see Tables 1 and 2). However, these molecular descriptors cannot be used to predict the order of elution of the molecular probes. Indeed, for the series phenanthrene/anthracene and chrysene/1,2-benzanthracene/triphenylene, similar  $V_{vdw}$  values, partition coefficient and correlation factors have been calculated, but different capacity factors are measured. These results demonstrate the limitation of these physico–chemical parameters to predict the order of elution of the PAH probes.

### 3.3. Capacity factors and polarizability $\alpha$ of PAH molecular probes

Fig. 2 shows the  $\log k'$  values versus the polarizability  $\alpha$  of the PAH molecular probes measured for both the silica–C<sub>18</sub> and silica–APS–PPyTS stationary phases with acetonitrile–water (90:10, v/v) as mobile phase. For both stationary phases, a linear increase of  $\log k'$  is observed as the polarizability  $\alpha$  of the PAH molecular probes increases (correlation factor  $r^2 > 0.958$ ). The polarizability  $\alpha$  of the PAHs can thus be used as a physico–chemical molecular descriptor to predict the order of elution of the probes. It is important to note that the slope of the linear relationships between  $\log k'$  and the polarizability  $\alpha$  is twice as large for the silica–APS–PPyTS than for the silica–C<sub>18</sub> stationary phase. This result clearly demonstrates a higher affinity of the PAH molecular probes towards the silica–APS–PPyTS stationary phase as their polarizability increases. Indeed, in addition to dispersive interactions (i.e., van der Waals interactions), the aromatic structures of both the PAH molecular probes and the polypyrrole backbone can induce  $\pi \rightarrow \pi^*$  interactions, and thus stronger interactions occur as the number of aromatic rings increases in the PAH

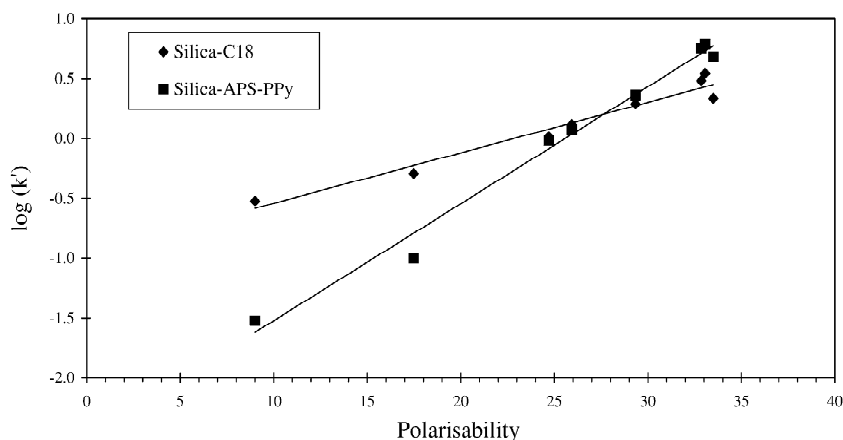


Fig. 2. Capacity factor logarithm ( $\log k'$ ) versus the polarisability  $\alpha$  of PAHs for silica- $C_{18}$  and silica-APS-PPyTS stationary phases with a mobile phase acetonitrile–water (90:10, v/v).

structure with the composite stationary phase [36]. To a lesser extent, the tosylate dopant species, incorporated in the polypyrrole during synthesis, can also interact with the PAH probes through  $\pi \rightarrow \pi^*$  interactions due to its aromatic character. The contribution of these two interactions explains the higher capacity factor observed for PAHs as the polarizability increase with the number of aromatic rings in the PAH structure with the silica-APS-PPyTS stationary phase [37].

### 3.4. Capacity factors and length-to-breadth ratio of PAH molecular probes

Fig. 3 shows  $k'$  versus  $L/B$  determined for the PAH molecular probes for both silica- $C_{18}$  and silica-APS-PPyTS stationary phases with acetonitrile–water (90:10, v/v) as mobile phase. For PAH molecular probes having similar molecular masses (i.e., phenanthrene/anthracene and triphenylene/1,2-benzanthracene/chrysene series) increasing chro-

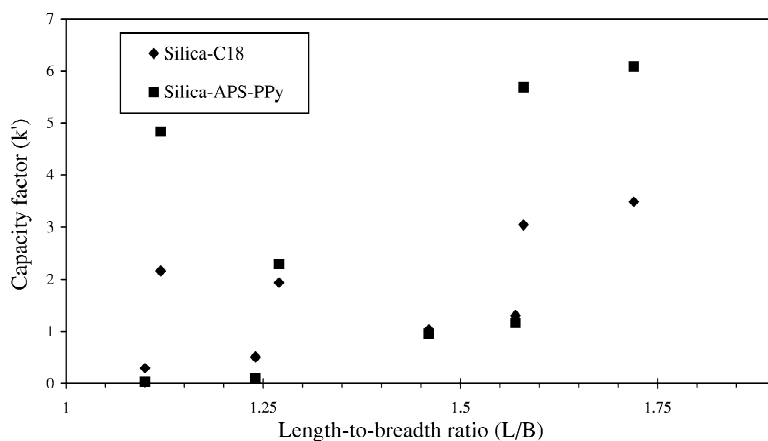


Fig. 3. Capacity factor ( $k'$ ) versus the length-to-breadth ratio ( $L/B$ ) of PAHs for silica- $C_{18}$  and silica-APS-PPyTS stationary phases with a mobile phase acetonitrile–water (90:10, v/v).

matographic retention is observed, for both the silica–APS–PPyTS and silica–C<sub>18</sub> stationary phases, as the *L/B* ratio of the probe increases, and thus, a stronger interaction takes place between the stationary phase and the molecular probes as expected. For the pairs benzene/triphenylene, naphthalene/pyrene and anthracene/1,2-benzanthracene, having similar *L/B* ratios, the order of elution is then governed by the molecular masses of the molecules, probes with lower molecular masses being eluted faster. The orders of elution observed for PAH molecular probes for both silica–C<sub>18</sub> and silica–APS–PPyTS stationary phases are in good agreement to previous results reported for silica, silanized-silica and polymer-modified silica stationary phases [38,39].

For PAHs having low molecular masses and low *L/B* ratios, lower capacity factors are observed for the silica–APS–PPyTS compared to the silica–C<sub>18</sub> stationary phase. However, for high-molecular-mass and high-*L/B*-ratio molecules, higher capacity factors are observed for the silica–APS–PPyTS compared to the silica–C<sub>18</sub> stationary phase. Moreover, for high-molecular-mass and low-*L/B*-ratio PAH molecular probes, higher capacity factors are observed for the silica–APS–PPyTS compared to the silica–C<sub>18</sub> stationary phase. These results clearly demonstrate a higher selectivity of the silica–APS–PPyTS stationary phase towards PAH molecular probes with high molecular masses.

Due to the coplanar structure, PAH molecules can adsorb parallel to the surface, and thus maximise its interaction with the stationary phase. Indeed, decreasing the approaching distance of the PAH molecule with the surface leads to increasing the strength

of interaction of the probe with the stationary phase. This phenomenon is of main importance in the case of a silica–APS–PPyTS stationary phase as both dispersive and  $\pi \rightarrow \pi^*$  interactions can occur between the PAH and the stationary phase. Indeed adsorption parallel to the surface favours  $\pi \rightarrow \pi^*$  interactions between the aromatic structures of the PAH molecular probes and the polypyrrole backbone. Moreover, the density of the  $\pi$ -electron cloud in the PAH molecule is related to its dimension. The higher the number of the  $\pi$ -electrons in a PAH molecule and the more compact the cloud of electron (i.e., the smaller the size of the molecule), the stronger is the specific interaction. This phenomenon is exacerbated in the case of pyrene and perylene probes.

### 3.5. Capacity factors of phenyl-PAH molecular probes

In order to evaluate the influence of the shape of the molecular probes on the chromatographic retention, *k'* values of a series of phenyl-PAH molecular probes have been determined. Fig. 1 presents the geometrical structures of the phenyl-PAH molecular probes used in this work. Table 3 reports on the physico-chemical properties of phenyl-PAH molecular probes (molecular mass and *L/B* ratio) and the capacity factor determined for both silica–C<sub>18</sub> and silica–APS–PPyTS stationary phases with acetonitrile–water (90:10, v/v) as mobile phase.

Lower capacity factors are observed for the silica–APS–PPyTS compared to the silica–C<sub>18</sub> stationary phase for all phenyl-PAH molecular probes. This

Table 3

Physico-chemical properties of phenyl-PAH molecular probes and capacity factors determined for silica–C<sub>18</sub> and silica–APS–PPyTS stationary phases with acetonitrile–water (90:10, v/v) as mobile phase

Molecular probe	Molecular mass (g mol <sup>-1</sup> )	<i>L/B</i> ratio	Capacity factor, <i>k'</i>	
			Silica–C <sub>18</sub> (Chrompack)	Silica–APS–PPyTS
Biphenyl	154.21	1.72	0.59	0.09
1-Phenylnaphthalene	204.27	1.27	1.00	0.12
<i>o</i> -Terphenyl	230.31	1.11	0.87	0.05
<i>m</i> -Terphenyl	230.31	1.47	1.03	0.28
<i>p</i> -Terphenyl	230.31	2.34	2.01	0.75
9,10-Diphenylanthracene	330.43	1.39	2.84	0.01
1,3,5-Triphenylbenzene	306.41	1.10	1.55	0.06



result demonstrates a higher affinity of the probes with the commercial silica- $C_{18}$  stationary phase.

It is important to note that for the series *o*-terphenyl/*m*-terphenyl/*p*-terphenyl, having similar molecular mass, the order of elution is then governed by the  $L/B$  ratio. Increasing chromatographic retention is observed as the  $L/B$  ratio increases, and this trend is noticed for both silica- $C_{18}$  and silica-APS-PPyTS stationary phases.

Comparison of the capacity factors determined for PAH and phenyl-PAH molecular probes having similar molecular masses or similar  $L/B$  ratios, clearly demonstrates the lower affinity of the phenyl-PAH molecular probes towards the stationary phases. For the pair terphenyl/benzanthracene (similar  $M_r$ ), the capacity factors measured for the phenyl-PAH is lower than those observed for the PAH molecular probe. In the same manner, for the series *m*-terphenyl/phenanthrene, 9,10-diphenylanthracene/anthracene and 1,3,5-triphenylbenzene/triphenylene (similar  $L/B$  ratios), the capacity factors of the phenyl-PAHs are lower than those of the PAH molecular probes, and this despite higher molecular masses of the phenyl-PAHs. In the case of the biphenyl molecular probe, despite its high  $L/B$  ratio and higher molecular mass compared to the naphthalene, this probe exhibits a lower capacity factor compared to naphthalene probe. It should be noted that for the pairs 1-phenylnaphthalene/pyrene and *o*-terphenyl/triphenylene (similar  $M_r$  and  $L/B$  ratio), lower chromatographic retentions are observed for the phenyl-PAH compared to the PAH molecular probes. These trends are observed for both silica- $C_{18}$  and silica-APS-PPyTS stationary phases with a similar mobile phase composition. The difference of the behaviour of the phenyl-PAH compared to PAH molecular probes, whatever the stationary phase, may be explained by the free rotation of the phenyl group. Indeed, this phenyl group induced a non-coplanar geometrical shapes of the phenyl-PAHs as illustrated in Fig. 1. Fig. 4 shows a schematic representation of the difference of behaviour observed for the phenyl-PAH and PAH probes. The steric hindrance induced by the rotation of the phenyl group limits the approaching distance of the phenyl-PAH compared to PAH probe having similar  $L/B$  ratio or molecular mass. Indeed, the strength of the dispersive interactions between molecules is propor-

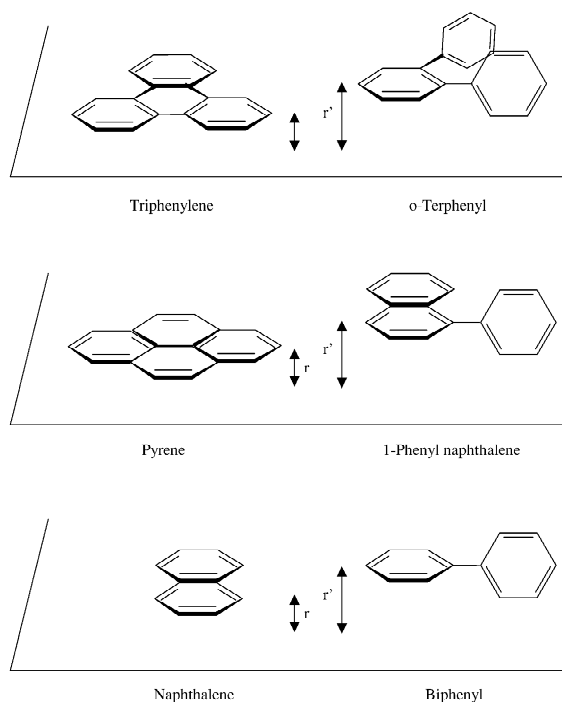


Fig. 4. Schematic representation of the difference of behaviour between PAHs and phenyl-PAHs towards the silica-APS-PPyTS stationary phase.

tional to  $1/r^6$  (where  $r$  is the distance between the two molecules), a slight variation of the distance of interaction of the solute with the stationary phase can lead to great changes in the strength of interaction between the molecules, and thus, affects the chromatographic retention of the given probes [36,40].

### 3.6. "Reverse" properties of the silica-APS-PPyTS stationary phase

The retention properties of PAH and phenyl-PAH molecular probes have been characterised depending the composition of the mobile phase for the silica-APS-PPyTS. Fig. 5 presents the capacity factors determined for molecular probes versus the volume fraction of acetonitrile in the mobile phase. Fig. 5 clearly demonstrates that decreasing the volume fraction of acetonitrile in the mobile phase leads to a relative increase of the capacity factors determined for both PAHs and phenyl-PAHs. Attempts to elute probes at lower acetonitrile volume

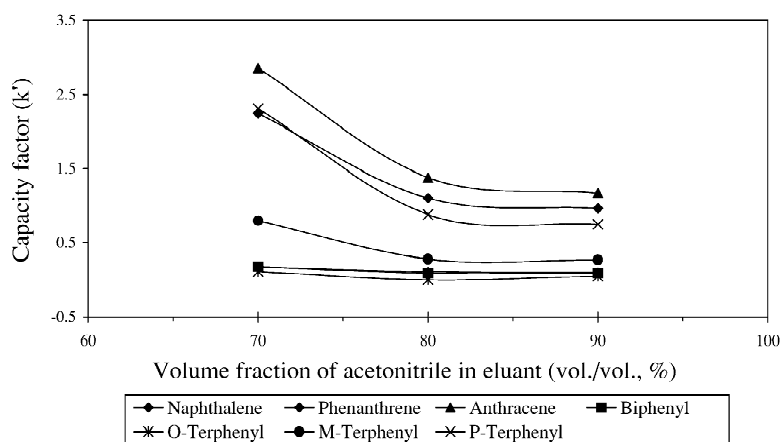


Fig. 5. Capacity factor versus the volume fraction of acetonitrile in the mobile phase for the silica-APS-PPyTS stationary phase.

fraction leads to too high retention of the probes or irreversibly adsorption of the probe on the stationary phase. Fig. 5 clearly shows that the chromatographic retention of a given PAH probes is highly dependent on the mobile phase composition. This result demonstrates the typical reversed-phase properties of the silica-APS-PPyTS stationary phase.

Snyder and co-workers demonstrated that for reverse  $\alpha$ -phase liquid chromatography, using a binary organic solvent-water mixture as mobile phase, the capacity factor of a given probe depends on the mobile phase composition as follows [41,42]:

$$\log k' = \log(k_{\text{water}}) - \rho C \quad (1)$$

where  $k_{\text{water}}$  represents the capacity factor of the given probe for pure water mobile phase,  $\rho$  is the volume fraction of organic solvent in the mobile phase mixture and  $C$  a constant depending on the nature and properties of the stationary phase under test. Therefore, increasing the volume fraction of acetonitrile in the mobile phase mixture induces a decrease of the capacity factor of a given probe.

PAHs and substituted PAHs are weakly soluble molecules in aqueous media [43]. Increasing the volume fraction of water in the mobile phase leads the PAH probe towards the less hydrophilic phase, and thus, towards the composite silica-APS-PPyTS stationary phase. This preferential segregation favours the interaction of the solute with the stationary phase and thus, leads to an increase of the chromatographic retention.


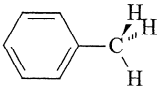
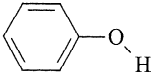
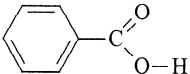
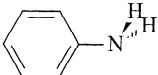
Determination of capacity factors of given PAH and substituted PAH probes depending the composition of the mobile phase demonstrates that, in addition the dispersive and  $\pi \rightarrow \pi^*$  interactions occurring between the probe and the stationary phase, hydrophobic interactions are of great importance, and thus, influence the chromatographic retention of PAH and phenyl-PAH probes.

### 3.7. "Acid-base" properties of the silica-APS-PPyTS stationary phase

In order to investigate the acid-base properties of the silica-APS-PPyTS stationary phase, the capacity factors of a series of mono-derivatized benzene molecular probes, bearing neutral, acidic or basic functional groups, have been determined. The molecular probes used are toluene, benzoic acid, phenol and aniline. Benzene is used as reference molecular probe. The structures and physico-chemical properties of these probes are presented in Table 4. As previously demonstrated, the molecular mass and ( $L/B$ ) ratio greatly influence the chromatographic properties of the probes. Thus, to limit the influence of these parameters, the molecular probes chosen have similar molecular masses and  $L/B$  ratios. Fig. 6 presents the capacity factors determined for benzene, toluene, benzoic acid, phenol and aniline for the silica-APS-PPyTS stationary phase with acetonitrile-water (90:10, v/v) as mobile phase.

A low capacity factor is determined for toluene,

Table 4  
Physico-chemical properties of neutral, acidic and basic benzene-derivative molecular probes

Molecular probe	Chemical structure	Molecular mass (g mol <sup>-1</sup> )	Boiling temperature (°C)	p <i>K</i>
Benzene		78.11	80	–
Toluene		92.14	111	–
Phenol		94.11	182	9.89
Benzoic acid		122.12	249	4.19
Aniline		93.13	184	9.37

similar to that observed for benzene. The neutral functional group (i.e., methyl) does not affect the retention of toluene. Indeed, as the benzene probe, toluene can only interact through dispersive interactions (i.e., Van der Waals interactions) and weak  $\pi \rightarrow \pi^*$  interactions with the stationary phase. Phenol and aniline molecular probes exhibit strong interac-

tions with the silica-APS-PPyTS stationary phase, and thus high capacity factors are determined for these probes. In the case of benzoic acid, no capacity factor could be determined. This result suggests a strong interaction or irreversible adsorption of the probe on the stationary phase. The non-elution of benzoic acid may be due to the high p*K* value of this

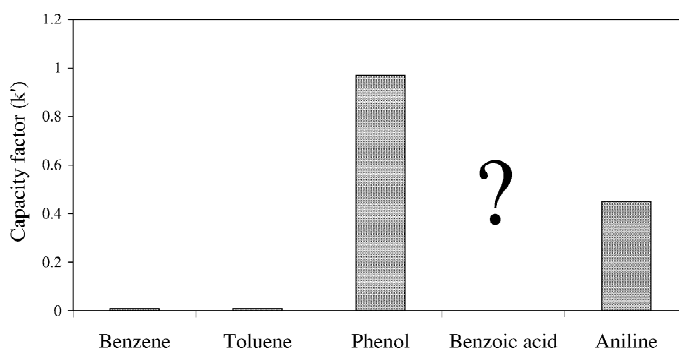


Fig. 6. Capacity factor for neutral, acidic and basic benzene-derivative molecular probes for silica-APS-PPyTS stationary phase with a mobile phase acetonitrile-water (90:10, v/v).

probe (see Table 4). Chriswanto et al. observed similar results for silica-PPyDS stationary phase [22].

Phenol and aniline, despite their low molecular masses and  $L/B$  ratios, exhibit similar capacity factors to those observed for phenanthrene. The high affinity of these probes can be explained by strong interaction induced by the acidic hydroxyl and basic amino groups, respectively, towards the stationary phase. Moreover, this result suggests that the stationary phase exhibits basic and acidic functional sites, respectively on its surface, and is thus able to interact with both acidic and basic molecular probes. Indeed, in addition to dispersive (i.e., Van der Waals interactions) and weak  $\pi \rightarrow \pi^*$  interactions, specific interactions, via hydrogen bonding and/or Lewis acid–base interactions, can occur between molecular probes and surface sites of the stationary phase having an antagonist character [36,44].

The high affinity of phenol and the non-elution of benzoic acid probes demonstrate that the polypyrrole overlayer masks the surface properties of silica substrate. Indeed, silica exhibits an acidic surface character, induced by the hydroxyl surface groups (isoelectric point of silica,  $pI \approx 2$ ), and thus silica does not interact with acidic molecular probes. In a similar manner, the high affinity of the aniline towards the stationary phase reflects the Lewis acidic character of the polypyrrole overlayer. Only polypyrrole, due to its amphoteric character, can interact with both acidic and basic molecular probes [18,21]. The high capacity factors determined for both phenol and aniline probes demonstrate that a polypyrrole overlayer homogeneously coats the silica–APS substrate. Previous works demonstrate that composite silica–APS–PPyTS particles exhibit a polypyrrole-rich surface [26–28]. Thus, the chromatographic properties of silica–APS–PPyTS stationary phase are governed by the intrinsic properties of the outer polypyrrole layer.

### 3.8. Correlation dependencies of chromatographic retention and molecular structure of PAHs for silica- $C_{18}$ and silica-APS-PPyTS stationary phases

Numerous attempts have been made to find correlation dependencies between the chromatographic

retention and the molecular structure of PAH molecules in liquid chromatography. In their outstanding and pioneering work, Snyder and Kirkland considered in detail the principle of additivity of the contribution of separate groups in the molecules in the chromatographic retention. To take into account the carbon atoms environment in the PAH molecule, they proposed the following linear correlation equation to describe the chromatographic retention of non-substituted PAHs in reversed-phase chromatography [45,46]:

$$\log k' = n_I X_1 + (n_{II} + n_{III}) X_2 + (n_\pi/L) X_3 + X_4 \quad (2)$$

where  $k'$  is the capacity factor of the PAH molecular probe;  $n_I$ ,  $n_{II}$  and  $n_{III}$  are the numbers of carbon atoms included in one, two or three aromatic cycles, respectively, in the PAH molecule;  $n_\pi$  is the number of  $\pi$ -electrons in the PAH molecule; and  $L$  is the length of the PAH molecule along the main axis according to Wise et al. (see Table 1) [9].  $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  are the equation coefficients. According to this model,  $n_I$ ,  $n_{II}$  and  $n_{III}$  reflect the differences in the specific interactions of the carbon atom with the stationary phase depending on their environment and  $(n_\pi/L)$  takes into account the mean density of  $\pi$ -electrons cloud depending the length of the PAH molecule. Table 5 reports on the number of carbon atoms of type  $n_I$ ,  $n_{II}$  and  $n_{III}$  and the number of  $\pi$ -electrons of PAH molecules used in this study.

Eq. (2) was applied to both silica- $C_{18}$  and silica-APS-PPyTS stationary phases with a mobile phase acetonitrile–water (90:10, v/v) for all PAH molecular probes, except for benzene and naphthalene, as

Table 5  
Number of carbon atoms of type I, II, and III and number of  $\pi$ -electrons in PAH molecular probes

Molecular probe	$n_I$	$n_{II}$	$n_{III}$	$n_\pi$
Benzene	6	0	0	6
Naphthalene	8	2	0	10
Phenanthrene	10	4	0	14
Anthracene	10	4	0	14
Pyrene	10	4	2	16
Chrysene	12	6	0	18
1,2-Benzanthracene	12	6	0	18
2,3-Benzanthracene	12	6	0	18
Perylene	12	4	2	20
Triphenylene	12	6	0	18

Table 6  
Values of the coefficients  $X_i$  and multiple correlation coefficient ( $r^2$ ) using Eq. (2) for both silica- $C_{18}$  and silica-APS-PPyTS stationary phases

Coefficients	Silica- $C_{18}$	Silica-APS-PPyTS
$X_1$	0.0836	0.19966
$X_2$	0.17186	0.18769
$X_3$	-0.70082	-0.33138
$X_4$	-0.61772	-2.32560
$r^2$	0.963	0.993

small capacity factors were determined. Table 6 reports the values of the coefficients  $X_i$  and the multiple correlation coefficient ( $r^2$ ) both silica- $C_{18}$  and silica-APS-PPyTS stationary phases. The high values of the correlation coefficient, for both stationary phases, clearly demonstrate that the model equation proposed by Snyder [45] describes quite well the chromatographic retention of PAH molecules in our chromatographic systems.

The silica-APS-PPyTS stationary phase is characterised by a higher value  $X_1$  compared to silica- $C_{18}$  stationary phase. This higher  $X_1$  value demonstrates the predominant contribution of the carbons the type I (characterised by higher electron density compared to carbon atoms of types II and III) to the retention of the PAH molecules in case of the silica-APS-PPyTS stationary phase. It should be noted that the coefficient  $X_3$  is a negative value, for both stationary phases, suggesting a repulsive interaction of the PAH probe with surface of the stationary phase induced by the  $\pi$ -electrons. However, a higher  $X_3$  value is observed for the silica-APS-PPyTS compared to silica- $C_{18}$  stationary phase. This result may be explained by the favourable  $\pi \rightarrow \pi^*$  interactions between the aromatic structures of the

PAH molecular probes and the polypyrrole backbone as previously demonstrated.

Table 7 presents experimental and calculated results of capacity factor logarithms according to Eq. (2) for PAH molecular probes for both silica- $C_{18}$  and silica-APS-PPyTS stationary phases. Table 7 shows that the calculated values of the capacity factor logarithms according to Eq. (2) and coefficients  $X_i$  (see Table 6) are close to the experimental values. These results demonstrate that Eq. (2) can be applied with good accuracy for the silica-APS-PPyTS stationary phase, and thus, can be used to predict capacity factors of given PAH probes.

#### 4. Conclusion

Composite silica-APS-PPyTS particles, having a high polypyrrole surface content, have been used as new stationary phase for liquid chromatography. The chromatographic properties of this polymeric-modified stationary phase was evaluated using a series PAHs and phenyl-PAHs as molecular probes. The silica-APS-PPyTS stationary phase exhibits a strong affinity and great selectivity towards these molecular probes depending on the molecular mass, polarisability, length-to-breadth ratio and geometrical structure. The reversed-phase character of the silica-APS-PPyTS stationary phase has been demonstrated, showing that hydrophobic interactions play an important role in retention mechanism of PAH probes. Acid-base properties of silica-APS-PPyTS stationary phase were evaluated using a series of benzene derivative probes. Silica-APS-PPyTS stationary phase exhibits amphoteric properties, characteristic of polypyrrole behaviour, and thus

Table 7  
Experimental (exp) and calculated (cal) results of capacity factor logarithms according to Eq. (2) for PAH molecular probes for both silica- $C_{18}$  and silica-APS-PPyTS stationary phases

Molecular probe	Silica- $C_{18}$		Silica-APS-PPyTS	
	Log $k'_{\text{exp}}$	Log $k'_{\text{cal}}$	Log $k'_{\text{exp}}$	Log $k'_{\text{cal}}$
Phenanthrene	0.01703	0.07070	-0.01773	0.02692
Anthracene	0.11727	0.06353	0.06818	0.02354
Pyrene	0.28780	0.28777	0.34242	0.34242
Chrysene	0.54283	0.51171	0.78462	0.76857
1,2-Benzanthracene	0.48287	0.51171	0.75435	0.76857
Triphenylene	0.33445	0.33661	0.68395	0.68578

confirms the high polypyrrole surface content of the composite particles. Finally, mathematical modelling gives good multi-linear correlation between the capacity factors measured by HPLC and molecular structure of PAH solutes. This study clearly demonstrates that silica–APS–PPyTS stationary phase exhibits interesting chromatographic properties and its potential application as new polymer-modified silica stationary phase for liquid chromatography.

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