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Phenylaminopropyl silica – a new specific stationary phase for high-performance liquid chromatography of phenols

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Abstract

The chromatographic properties of a new stationary phase, phenylaminopropyl silica (PhA-silica), containing phenylaminopropyl residues covalently bonded to the silica surface were studied. The presence of secondary amino groups, phenyl rings and alkyl linkers in the attached molecule makes it especially suitable for the separation of phenols by mixed mode retention mechanism including a combination of hydrogen-bonding, hydrophobic, electrostatic and $\pi-\pi$ interactions with the stationary phase. The effects of mobile phase pH, ionic strength, nature and concentration of organic modifier on the retention of phenols on PhA-silica were investigated under conditions of reversed-phase HPLC. To elucidate the role of the amino group in the attached molecule in retention of phenols the selectivity of PhA-silica was compared with that obtained for phenylpropyl silica in the framework of a linear solvation energy relationship (LSER) model. The isocratic separation of phenol, and its nine methyl-, chloro- and nitro-substituted derivatives was achieved on a 150×4.6 mm I.D. chromatographic column packed with 7 μm particles of PhA-silica.

Keywords: Phenylaminopropyl silica; Stationary phases, LC; Linear solvation energy relationships; Mobile phase composition; Phenols

1. Introduction

The number of various substituted phenols that must be monitored in natural and industrial waters is constantly increasing, so in spite of a large number of existing stationary phases the interest in the design of sorbents for separation of phenols by high-performance liquid chromatography (HPLC) is still very high. Reversed-phase (RP) HPLC on different types of ODS columns with UV detection or with electrochemical detection is the most common way for the determination of phenols in different water samples. The retention of phenols in this chromatographic system is mainly due to hydrophobic interactions while the selectivity of their separation can be regulated by change in the pH of the eluent, which has an effect on the ionic state of phenols and hence on their ability to be retained. However, adjustment of the pH of the eluent does not solve all of present analytical tasks related to their separation and determination.

The retention of phenols on the stationary phase is governed mainly by hydrophobic interactions, but also may involve other mechanisms such as hydro-

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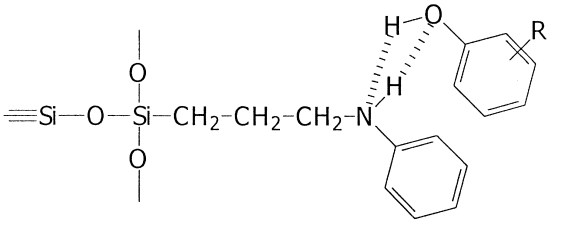
gen bonding, anion-exchange, π–π interactions and more complicated mechanisms such as complexation with transition metals on metal-loaded stationary phases and size-specific chromatography. So, many different stationary phases utilising a mixed-mode mechanism such as phenyl- [1–3], alkenyl- [4], cyano- [2,4–7], amino- [2,5,7,8], diol- [4,5], fluorocarbon-diamine [8], cyclodextrins [9,10], iron(III)–8-hydroxyquinolinol complex [11], modified silica materials, molecular imprinted polymers [12], porous graphitic carbon [13], hypercrosslinked polystyrene [14,15] and common polystyrene–divinylbenzene (PS–DVB) resins [16] were proposed to achieve the desired improvements in the selectivity of separation of phenols.

It is important to note that most of the above mentioned phases have been used with polar eluents. Use of polar mobile phases makes column handling easier, its contamination by adsorption of non-polar, highly hydrophobic constituents of the sample can be overcome by washing the column with less polar or more lipophilic solvent. Another important advantage of a polar mobile phase is its high compatibility with aqueous samples.

Hydrophobic effects are responsible for the separation of phenols on alkyl silica-based stationary phases in RP-HPLC. The selectivity of reversed-phase separations can be improved by introduction of phenyl groups into bonded molecules providing π–π interactions between the stationary phase and phenols. Compared to ODS, the phenyl silica proved to separate early eluting chlorophenols with the same efficiency, but the retention of polychlorophenols was much less [3]. An additional change in the selectivity of the stationary phase can be achieved by introduction of polar functional groups like diol, amino and cyano into the bonded non-polar layer. Amino, hydroxy and cyano groups are responsible for dipole–dipole, electrostatic interactions and H-bonding. The comparison of ODS and aminopropyl silica for the separation of chlorophenols under reversed-phase chromatographic conditions showed the advantages of ODS for both group and individual separations [1]. At the same time the long-chain alkylnitrile bonded to silica demonstrated excellent selectivity in the separation of xylenols [6].

The original idea of the present paper is to combine different possible sorbate–sorbent interactions in one stationary phase and to get the structure of such a phase in a form attuned maximally to the structure of phenols. For this purpose the novel stationary phase – phenylaminopropyl silica (PhA-silica):



was synthesised and studied. The special structure of the attached molecules provides a mixed mode separation mechanism that in some respect is very similar to the basic idea of the three-point chiral-recognition mechanism [17] in the most specific chromatographic systems like chiral bonded phase chromatography. The phenyl group in PhA-silica is responsible for π–π interactions and together with the $-(CH_2)_3-$ spacer provides sufficient hydrophobic properties. The presence of a secondary amino group attached to the silica molecule can be responsible for hydrogen bonding with non dissociated phenols and for anion-exchange with dissociated species under acid eluent. Thus the structure of the phenylaminopropyl moiety is ''complementary'' to the structure of phenols.

So, the main aim of the present paper is to define the selectivity of PhA-silica to phenols and to study the mechanism of their retention. To evaluate the effect of the amino group on the retention of phenols on PhA-silica comparative experiments were also performed on a column packed with phenylpropyl silica (Ph-silica).

2. Experimental

2.1. Apparatus

The isocratic HPLC system consisted of a Model 101A pump, a Model 210 injection valve (both from Altex, Berkeley, CA, USA) equipped with a 100-μl sample loop and a Model 2238 Uvicord spectrophotometric detector (LKB, Bromma, Sweden) set at 280 nm for detection of phenols and at 254 nm for

detection of other benzene derivatives. A stainless steel guard column (40×3 mm I.D.) followed by a stainless steel column (150×4.6 mm I.D.) both slurry packed with PhA-silica from a 2-propanol–water mixture and a commercially available stainless steel Si 100 Polyol Phenyl 5 μm 250×4.6 mm I.D. column (Serva, Heidelberg, Germany) were used in the experiments.

2.2. Reagents

Acetonitrile (HPLC grade, E.Merck, Darmstadt, Germany), methanol (analytical grade, Reakhim, Moscow, Russia), solutions of sodium acetate trihydrate (HPLC grade, J.T. Baker, Phillipsburg, NJ, USA), acetic acid (HPLC grade, Fisher, USA) and sodium nitrate (analytical grade, Reakhim) in distilled water were used for preparation of mobile phases. All tested compounds were of analytical grade.

2.3. Preparation of PhA-silica

The PhA-silica was prepared by treatment of silica particles with [3-(phenylamino)propyl]trimethoxysilane in toluene. [3-(Phenylamino)propyl]-trimethoxysilane and toluene (both from Fluka, Deisenhofen, Germany) were used for the preparation of PhA-silica. A narrow fraction of small size irregular particles (7 μm) of silica KSK-G (Reakhim, Nizniy Novgorod, Russia) with a specific surface area of 300 m^2/g was used as a matrix. The mixture of 25 g of KSK-G silica gel and 25 ml of [3-(phenylamino)propyl]trimethoxysilane in 320 ml of toluene was refluxed under stirring for 24 h. The bonded phase was filtered on glass filter, washed with toluene, diethyl ether and water and dried.

2.4. Data analysis

Multivariate linear regression analysis and statistical tests were performed using SPSS 7.52 software (SPSS, Chicago, IL, USA). The solute descriptors were taken from several sources [18–20] and are summarised in Tables 4 and 6.

3. Results and discussion

There are three main types of sorbate–sorbent interactions that can contribute to the retention of phenols on phenylaminopropyl silica: hydrophobic, π–π and electrostatic. Depending on the separation conditions the electrostatic interactions between the amino group of PhA-silica and dissociated phenols may be considered as either ion-exchange or hydrogen bonding, which is considered [21] an exceptional case of short-range electrostatic interactions. As there is a big difference in strength of these interactions, the separation selectivity of PhA-silica can be regulated by change of pH of the eluent. The nature and concentration of organic solvent in the mobile phase affect the strength of hydrophobic and π–π interactions. To evaluate the chromatographic properties of PhA-silica and the optimum separation conditions for phenols the effects of the nature and concentration of organic modifier, pH and ionic strength of the mobile phase were studied.

3.1. Effect of pH of the eluent

PhA-silica is expected to have not only hydrophobic and π–π, but also electrostatic interactions between bonded phenylaminopropyl groups and separated phenols. The pK_a value of phenylaminopropyl groups in PhA-silica is expected to be in the range 2.5–3.1 [22]. Thus, electrostatic attraction between the protonated amino group of PhA-silica and the dissociated hydroxy group of phenols is possible for those phenols that are dissociated at pH close to 3.

To understand the role of the secondary amino group of PhA-silica in the retention of phenols the dependence of the capacity factor (k') on pH of the eluent in the range from 3.5 to 6.0 was obtained for seven phenols and compared with those for Ph-silica (Fig. 1). It is curious that the retention of non-dissociated under the studied pH range phenols is practically equal on both polar PhA-silica having amino group and non-polar Ph-silica stationary phases. Such a result is less expected especially at pH 3.5, where PhA-silica is positively charged due to the protonation of attached phenylaminopropyl groups. A possible explanation of this phenomenon is the blocking or binding of the secondary amino

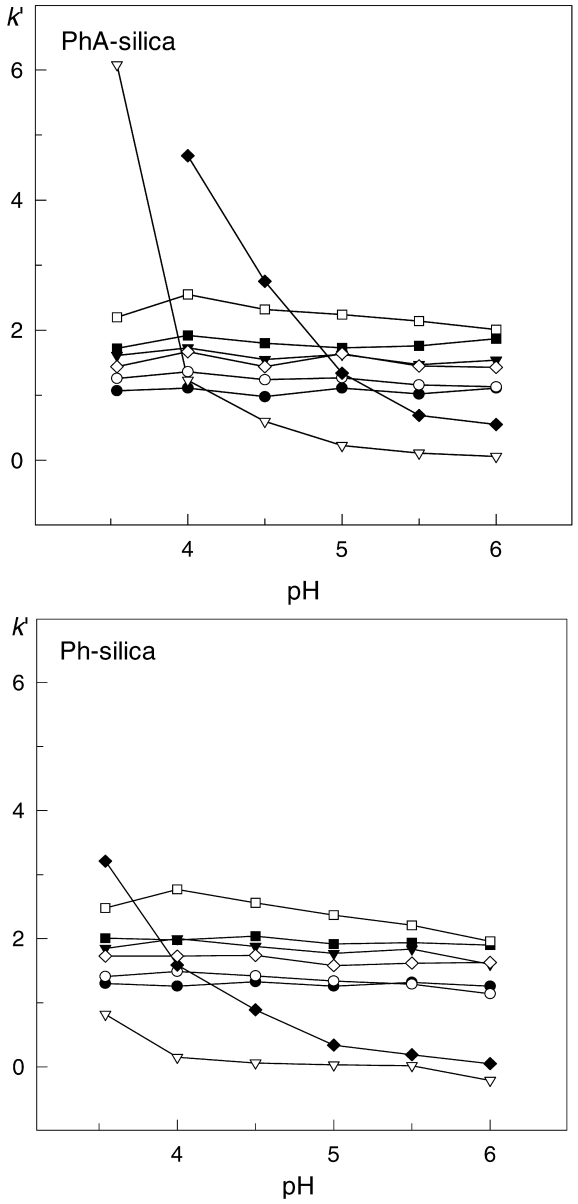


Fig. 1. The effect of pH on retention (k') of phenols on PhA-silica and Ph-silica. Eluent: acetonitrile–5 m*M* sodium acetate buffer (50:50, v/v). Phenol (●), *p*-nitrophenol (○), *o*-nitrophenol (▼), 2,4-dinitrophenol (▽), 2,4-dichlorophenol (■), 2,4,5-trichlorophenol (□), pentachlorophenol (♦) and 2,5-dimethylphenol (◇).

groups by residual silanols at the surface of PhA-silica. Thus, amino groups become unapproachable for the most of studied phenols and do not impact on their retention.

The difference between the two stationary phases is evident by higher retention of 2,4-dinitrophenol and pentachlorophenol at a pH of about 3.5. These two phenols are stronger acids (Table 1) than silanol groups (pK_a is about 6.5 [24]) so they can successfully compete with the latter for binding with amino groups. So, the high retention of dissociated pentachlorophenol and 2,4-dinitrophenol on PhA-silica can be accounted for by the electrostatic interaction between the oppositely charged amino group and the $\pi-\pi$ interaction between the phenyl groups of bonded and solute molecules.

For the both stationary phases the pH of the eluent affects the retention of only 2,4-dinitrophenol and pentachlorophenol which can dissociate in a pH range from 3.5 to 6.0 while the retention of other phenols is practically constant (Table 2).

3.2. Effect of organic modifier concentration in the eluent

Acetonitrile and methanol are the most common organic modifiers of eluent in RP-HPLC. They have different polarity and H-bond donating ability (Table 3). Methanol is less lipophilic than acetonitrile (log *P* values are -0.723 and -0.307, respectively [21]), so at the same concentration of these organic solvents in the mobile phase the retention of phenols should be higher in the case of methanol-containing eluent. On the other hand, methanol, being a strong H-bond donor, can compete with phenols for hydrogen bonding with the amino group of PhA-silica. So, the following non-equality must be true:

$$(k'_{\mathrm{MeOH}}/k'_{\mathrm{MeCN}})_{\mathrm{Ph\text{-}silica}} > (k'_{\mathrm{MeOH}}/k'_{\mathrm{MeCN}})_{\mathrm{PhA\text{-}silica}}$$

However, practically for all phenols studied the

Table 1
Dissociation constants of studied phenols [23]

Compound	pK_a	Compound	pK_a
2,4-Dinitrophenol	4.09	3-Methyl-4-chlorophenol	9.55
Pentachlorophenol	5.12	Phenol	9.99
2,4,5-Trichlorophenol	6.94	*p*-Methoxyphenol	10.10
p-Nitrophenol	7.08	*p*-Cresol	10.26
o-Nitrophenol	7.23	3,4-Dimethylphenol	10.36
2,4-Dichlorophenol	7.89	2,5-Dimethylphenol	10.41
4-Chlorophenol	9.41		

Table 2
Capacity factors (k') of phenols on PhA-silica and Ph-silica at different pH[a]

Compound	pH					
	3.5	4.0	4.5	5.0	5.5	6.0
Capacity factors on PhA-silica						
Phenol	1.07	1.11	0.98	1.11	1.02	1.11
o-Nitrophenol	1.61	1.73	1.55	1.63	1.47	1.54
p-Nitrophenol	1.26	1.36	1.24	1.27	1.16	1.13
p-Methoxyphenol	1.02	1.13	1.05	1.02	1.01	1.03
4-Chlorophenol	1.41	1.51	1.38	1.42	1.41	1.50
2,4-Dichlorophenol	1.72	1.92	1.80	1.73	1.76	1.87
4-Chloro-3-methylphenol	1.59	1.76	1.68	1.57	1.60	1.59
p-Cresol	1.25	1.46	1.37	1.39	1.24	1.23
3,4-Dimethylphenol	1.40	1.61	–	–	1.41	1.38
2,5-Dimethylphenol	1.44	1.67	1.44	1.64	1.45	1.43
2,4-Dinitrophenol	6.08	1.23	0.60	0.23	0.11	0.06
Pentachlorophenol	–	4.68	2.75	1.34	0.69	0.55
2,4,5-Trichlorophenol	2.20	2.55	2.32	2.24	2.14	2.01
Capacity factors on Ph-silica						
Phenol	1.30	1.26	1.33	1.26	1.32	1.26
o-Nitrophenol	1.85	2.00	1.88	1.77	1.84	1.60
p-Nitrophenol	1.41	1.49	1.42	1.34	1.29	1.14
p-Methoxyphenol	1.19	1.25	1.23	1.18	1.19	1.19
4-Chlorophenol	1.64	1.59	1.67	1.59	1.60	1.59
2,4-Dichlorophenol	2.01	1.98	2.04	1.92	1.94	1.90
4-Chloro-3-methylphenol	1.90	1.77	1.92	1.82	1.83	1.82
p-Cresol	1.47	1.48	1.48	1.39	1.40	1.41
3,4-Dimethylphenol	1.68	1.67	1.67	1.54	1.57	1.57
2,5-Dimethylphenol	1.73	1.73	1.74	1.58	1.62	1.63
2,4-Dinitrophenol	0.82	0.15	0.06	0.03	0.02	−0.21
Pentachlorophenol	3.21	1.59	0.89	0.34	0.19	0.05
2,4,5-Trichlorophenol	2.48	2.77	2.56	2.37	2.21	1.96

[a] Mobile phase: acetonitrile–5 m*M* sodium acetate buffer (50:50, v/v).

opposite effect was found. For mobile phases with 30% (v/v) of organic solvent the ratios of k'_{MeOH}/k'_{MeCN} were equal to 1.02 and 1.11 for phenol; 1.09 and 1.52 for *p*-nitrophenol; 1.84 and 9.81 for 2,4-dinitrophenol, 1.73 and 2.46 for 2,4,5-trichlorophenol; 1.33 and 1.38 for 2,5-dimethylphenol on Ph-silica and PhA-silica, respectively. The possible explanation is in blocking of the residual silanols at the surface of stationary phases by methanol molecules, which have a higher hydrogen bonding ability. This supposition is in accordance with the results obtained by Lu and Rutan for 1-propanol, the addition of which to the eluent markedly changed the solvatochromic parameters of the C_{18} bonded silica [26]. It disrupts the hydrogen bonds between the amino groups of PhA-silica and silanols, so the electrostatic interactions of phenols with the ''released'' amino group of PhA-silica become possible, providing a growth of retention. One more proof in favour of above explanation is the unexpectedly high retention of 2,4-dinitrophenol and pentachlorophenol on PhA-silica obtained with methanol-containing eluents as compared to acetonitrile (Figs. 2 and 3). For the whole range of methanol concentrations

Table 3
Properties of the eluent components according to Kamlet et al. [25]

Compound	π^*[a]	β[a]	α[a]
Acetonitrile	0.75	0.31	0.19
Methanol	0.60	0.62	0.93
Water	1.09	0.18	1.17

[a] π^*: Solvent polarity/polarizability, α: overall hydrogen bond donor acidity, β: overall hydrogen bond acceptor basicity.

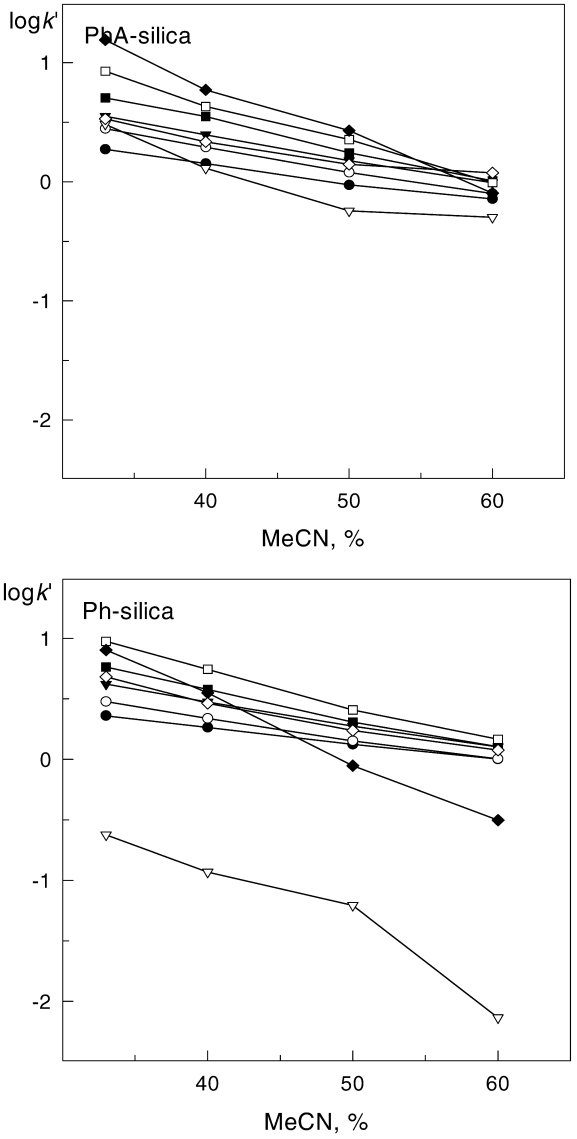


Fig. 2. The effect of acetonitrile concentration (v/v, %) on retention (log k') of phenols on PhA-silica and Ph-silica. Eluent: acetonitrile–5 m*M* sodium acetate buffer, pH 4.5. Solutes labelled as in Fig. 1.

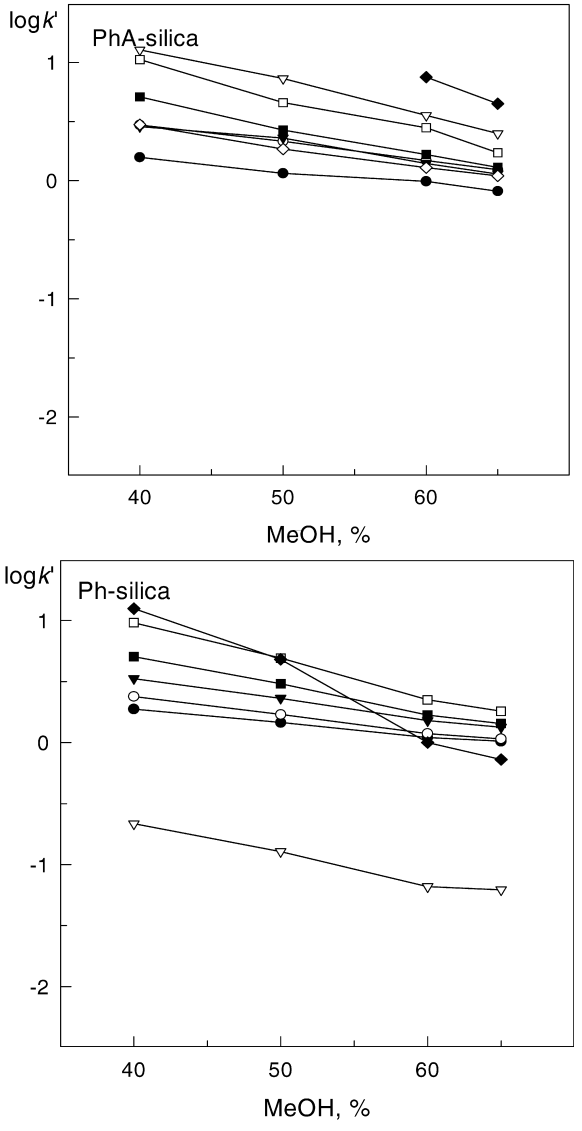


Fig. 3. The effect of methanol concentration (v/v, %) on retention (log k') of phenols on PhA-silica and Ph-silica. Eluent: methanol–5 m*M* sodium acetate buffer, pH 4.5. Solutes labelled as in Fig. 1.

2,4-dinitrophenol was retained more strongly than all the other studied phenols, except pentachlorophenol. It should be noted that the separation selectivity of phenols on Ph-silica does change slightly with organic modifier change. The best separations of the studied phenols at pH 4.5 can be achieved with 35% (v/v) acetonitrile on both PhA-silica and Ph-silica.

The decrease in the retention of phenols with increase of organic modifier concentration in the eluent is sharper for 2,4-dinitrophenol and pentachlorophenol for both stationary phases and for both organic modifiers. This can be due to the partial dissociation of these phenols at pH 4.5, which is close to their pK_a values.

3.3. Effect of ionic strength of the eluent

For additional evaluation of the electrostatic interactions between 2,4-dinitrophenol and pentachlorophenol and phenylaminopropyl groups of PhA-silica, retention of phenols was studied under high ionic strength of the eluent, created by sodium nitrate. High ionic strength ($\mu > 0.1$) tends to suppress the electrostatic interactions between solute and stationary phase. On the other hand it may cause the salting out effect in reversed-phase chromatographic systems. The first factor leads to decrease of the retention of phenols, especially acidic ones, on PhA-silica. The second increases the retention under reversed-phase conditions. It is difficult to find strict regularity in changes of capacity factors between studied phenols under different ionic strengths and at various concentrations of organic modifier in the eluent. To make these regularities more expressive it is reasonable to use factor *f*, which characterises the relative change in the retention of phenols while passing to the eluent with high ionic strength:

$$f = (k'_{\mu=0.2} - k'_{\mu\sim0})/k'_{\mu\sim0}$$

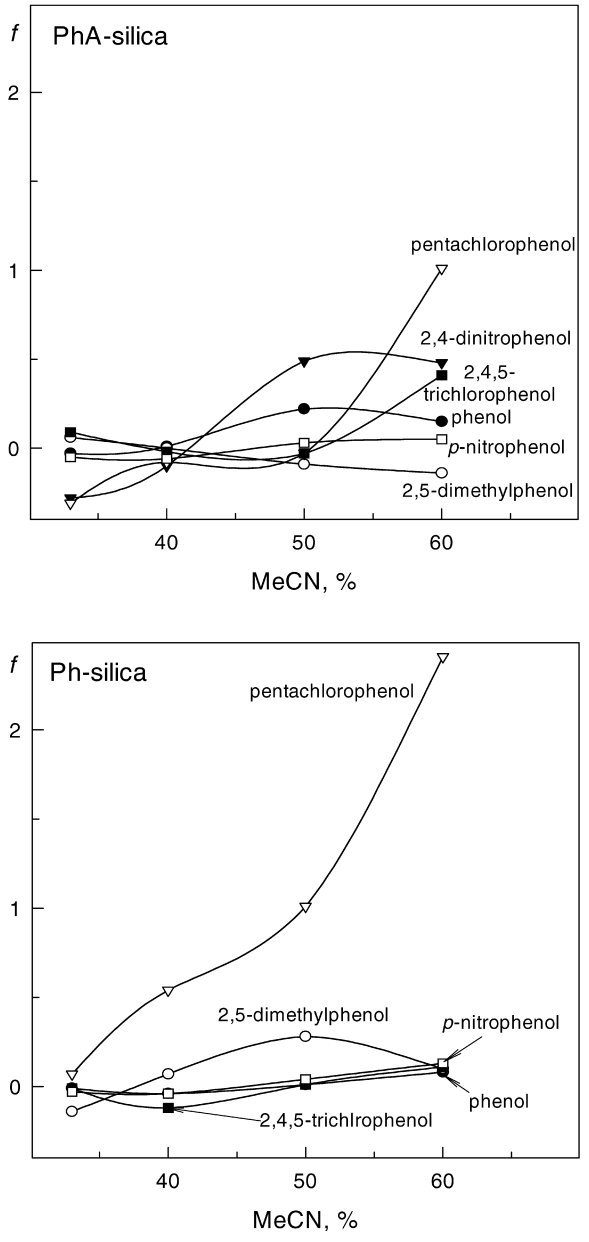


Fig. 4. The effect of ionic strength on the relative retention $[k'_{(\mu=0.2)}/k'_{(\mu\to0)}]$ of phenols on PhA-silica and Ph-silica plotted vs. concentration of acetonitrile in the eluent. Eluent: acetonitrile–5 m*M* sodium acetate buffer, pH 4.5.

When factor *f* is greater than zero, the retention of phenols is higher in the case of eluent with high ionic strength. The effect of ionic strength is more noticeable for the retention of 2,4-dinitrophenol and pentachlorophenol on both stationary phases and for the retention of 2,4,5-trichlorophenol on PhA-silica (Fig. 4). In the case of Ph-silica, an increase of ionic strength causes an abrupt increase of the retention of 2,4-dinitrophenol ($5.9 < f < 65$) and pentachlorophenol ($0.1 < f < 2.4$) at any studied concentration of acetonitrile in the eluent. In the case of PhA-silica, factor *f* for 2,4-dinitrophenol, pentachlorophenol and 2,4,5-trichlorophenol is negative at concentrations of acetonitrile below 40 and 50% (v/v). It means that ionic strength causes a decrease of retention of these phenols under such conditions. It can be accounted for the suppression of electrostatic interactions of these phenols with the stationary phase amino groups. At higher concentrations of acetonitrile in the eluent factor *f* for 2,4-dinitrophenol, pentachlorophenol and 2,4,5-trichlorophenol is positive, but still it is not so high ($f < 1$) as in the case of Ph-silica.

It is well known that the pK_a values of weak acids increase with the increase of organic solvent content in the eluent [27]. It means that in the studied chromatographic system pH of the eluent created by the sodium acetate buffer decreases and the pK_a

values of the studied phenols increase with concentration of acetonitrile in the eluent. Thus, the dissociation degrees of 2,4-dinitrophenol and pentachlorophenol decrease, causing a growth of hydrophobic interaction impact on retention.

3.4. Linear solvation energy relationships for PhA-silica and Ph-silica

To outline the effect of the amino group in PhA-silica on retention of non-dissociated phenols the LSER model was applied. This model proved to be very useful for characterisation of different stationary phases for RP-HPLC [28–34]. The unique advantage of this approach is ability to measure independently the contribution of each possible type of molecular interactions in retention. According to Oumada et al. [18] the retention of solute can be described by the equation:

$$\log k' = c + rR_2 + s\pi^*_2 + a\sum\alpha^{H}_2 + b\sum\beta^{H}_2 + vV_x \quad (1)$$

where c is the intercept, R_2 is an excess molar refraction, π^*_2 is the solute polarity/polarizability, $\Sigma\alpha^{H}_2$ is the solute overall hydrogen bond donor (HBD) acidity, $\Sigma\beta^{H}_2$ is the solute overall hydrogen bond acceptor (HBA) basicity, V_x is a McGowan characteristic volume of the solute. Solute descriptors are derived from equilibrium measurements on the solutes themselves such as GC or LC data, water–solvent partition coefficients and data relating to molecular structure. Nowadays, solute descriptors are available for many compounds [19,20]. The coefficients in Eq. (1) can be determined by multivariate regression analysis and are characteristics of the phase investigated under certain conditions. Special attention should be paid to the fact, that each coefficient reflects the difference in complementary property of the mobile and stationary phases. r is a

Table 4
Descriptors of test solutes and their retention on PhA-silica and Ph-silica[a]

Compound	R_2	π^*_2	$\Sigma\alpha^{H}_2$	$\Sigma\beta^{H}_2$	V_x	PhA-silica		Ph-silica	
						pH 3.54	pH 6.00	pH 3.54	pH 6.00
Benzene	0.610	0.52	0	0.14	0.7164	0.448	0.505	0.538	0.555
Toluene	0.601	0.52	0	0.14	0.8573	0.569	0.612	0.645	0.683
Ethylbenzene	0.613	0.51	0	0.15	0.9982	0.664	0.732	0.802	0.802
p-Xylene	0.613	0.52	0	0.16	0.9982	0.680	0.732	0.770	0.796
n-Propylbenzene	0.604	0.50	0	0.15	1.1391	0.805	0.906	0.952	0.945
i-Propylbenzene	0.602	0.49	0	0.16	1.1391	0.770	0.866	0.888	0.915
Naphtalene	1.340	0.92	0	0.20	1.0854	0.741	0.799	0.815	0.821
Anthracene	2.290	1.34	0	0.26	1.4540	1.096	1.167	1.088	1.101
Fluorene	1.588	1.03	0	0.20	1.3565	0.988	1.028	1.017	1.012
o-Dichlorobenzene	0.872	0.78	0	0.04	0.9612	0.724	0.780	0.804	0.812
Bromobenzene	0.882	0.73	0	0.09	0.8914	0.639	0.703	0.724	0.735
Nitrobenzene	0.871	1.11	0	0.28	0.8906	0.451	0.487	0.522	0.531
p-Nitrotoluene	0.870	1.11	0	0.28	1.0315	0.557	0.610	0.630	0.636
Anisole	0.708	0.75	0	0.29	0.9160	0.491	0.488	0.540	0.545
Benzonitrile	0.742	1.11	0	0.33	0.8711	0.362	0.336	0.444	0.453
Methylbenzoate	0.733	0.85	0	0.46	1.0726	0.398	0.443	0.505	0.514
Acetophenone	0.818	1.01	0	0.48	1.0139	0.316	0.340	0.395	0.399
Benzophenone	1.447	1.50	0	0.50	1.4808	0.734	0.788	0.791	0.803
Benzyl alcohol	0.803	0.87	0.33	0.56	0.9160	0.302	0.321	0.388	0.379
Benzaldehyde	0.818	1.00	0	0.39	0.8730	0.290	0.324	0.384	0.381
Dimethylphtalate	0.780	1.41	0	0.88	1.4288	0.380	0.403	0.462	0.470
α-Naphthol	1.520	1.05	0.61	0.37	1.1440	0.514	0.549	0.547	0.565
β-Naphthol	1.520	1.08	0.61	0.40	1.1440	0.470	0.494	0.480	0.516
Phenol	0.805	0.89	0.60	0.31	0.7751	0.165	0.165	0.221	0.252

[a] Eluent: acetonitrile–5 m*M* sodium acetate buffer (40:60, v/v), pH 3.54 and 6.00.

Table 5
Regression coefficients and regression statistics of LSER equations for PhA-silica and Ph-silica at different pH values of eluent[a]

Stationary phase and eluent pH	Coefficients						Statistics[b]				Relative coefficients			
	v	*r*	*b*	*a*	*s*	*c*	*R*	SE	*F*	*n*	*r*/*v*	*b*/*v*	*a*/*v*	*s*/*v*
PhA-silica,	0.80	0.17	−0.70	−0.26	−0.20	−0.02	0.987	0.04	137	24	0.21	−0.88	−0.32	−0.25
pH 3.54	(0.07)	(0.05)	(0.10)	(0.05)	(0.07)	(0.06)								
PhA-silica,	0.89	0.15	−0.79	−0.28	−0.22	−0.01	0.987	0.05	133	24	0.17	−0.88	−0.31	−0.25
pH 6.00	(0.08)	(0.05)	(0.10)	(0.06)	(0.07)	(0.06)								
Ph-silica,	0.86	0.09	−0.71	−0.28	−0.23	0.09	0.985	0.04	117	24	0.11	−0.83	−0.32	−0.26
pH 3.54	(0.08)	(0.05)	(0.10)	(0.06)	(0.07)	(0.06)								
Ph-silica,	0.87	0.09	−0.74	−0.25	−0.22	0.10	0.988	0.04	145	24	0.10	−0.85	−0.28	−0.25
pH 6.00	(0.07)	(0.05)	(0.09)	(0.05)	(0.06)	(0.05)								

[a] Eluent: acetonitrile–sodium acetate buffer (50:50, v/v).
[b] *R*: Overall correlation coefficient; SE: standard error of the estimate; *F*: *F*-change (change statistics); *n*: number of solutes; numbers in parentheses indicate the standard deviation in the coefficient.

measure of the ability of the phase to interact with the solute's n- and π-electron pairs; *s* describes phase polarity/polarizability; *a* and *b* are the measures of the phase hydrogen bond basicity and acidity, respectively; *v* measures phase lipophilicity [29]. An important fact to remember is that parameters describing phase properties depend on the surface concentration of bonded groups. As far as there is no precise information on this matter it is better to compare relative parameters: r/v, s/v, a/v and b/v.

Twenty-four analytes, providing a wide range of

Table 6
Descriptors of phenols and their retention on PhA-silica and Ph-silica[a]

Compound	R_2	π^*_2	$\Sigma\alpha^H_2$	$\Sigma\beta^H_2$	V_x	PhA-silica		Ph-silica	
						pH 3.54	pH 6.00	pH 3.54	pH 6.00
o-Nitrophenol	1.015	1.05	0.05	0.37	0.9493	0.426	0.457	0.402	0.495
						(0.377)[b]	(0.389)	(0.453)	(0.445)
p-Nitrophenol	1.070	1.72	0.82	0.26	0.9493	0.181	0.230	0.115	0.244
						(0.280)	(0.268)	(0.335)	(0.321)
p-Methoxyphenol	0.900	1.17	0.57	0.48	0.9747	0.192	0.231	0.173	0.272
						(0.121)	(0.145)	(0.191)	(0.232)
2,4-Dichlorophenol	0.960	0.84	0.53	0.19	1.0199	0.519	0.590	0.504	0.614
						(0.481)	(0.501)	(0.537)	(0.549)
3-Methyl-4-chlorophenol	0.920	1.02	0.65	0.23	1.0384	0.432	0.502	0.417	0.528
						(0.416)	(0.453)	(0.477)	(0.513)
p-Cresol	0.820	0.87	0.57	0.32	0.9160	0.305	0.358	0.303	0.399
						(0.245)	(0.265)	(0.335)	(0.343)
3,4-Dimethylphenol	0.830	0.86	0.56	0.39	1.0569	0.374	0.435	0.380	0.475
						(0.315)	(0.347)	(0.405)	(0.421)
2,5-Dimethylphenol	0.840	0.79	0.54	0.37	1.0569	0.409	0.472	0.415	0.511
						(0.332)	(0.367)	(0.437)	(0.137)
2,4-Dinitrophenol	1.200	1.50	0.10	0.55	1.1235	0.367	0.389	0.310	0.418
						(1.162)	(−0.914)	(0.255)	(−1.194)
Pentachlorophenol	1.220	0.87	0.96	0.01	1.3871	0.866	0.994	0.824	0.977
						(1.238)	(0.224)	(0.896)	(0.002)

[a] Eluent: acetonitrile–5 m*M* sodium acetate buffer (40:60, v/v), pH 3.54 and 6.00.
[b] Numbers in parentheses indicate the experimental data.

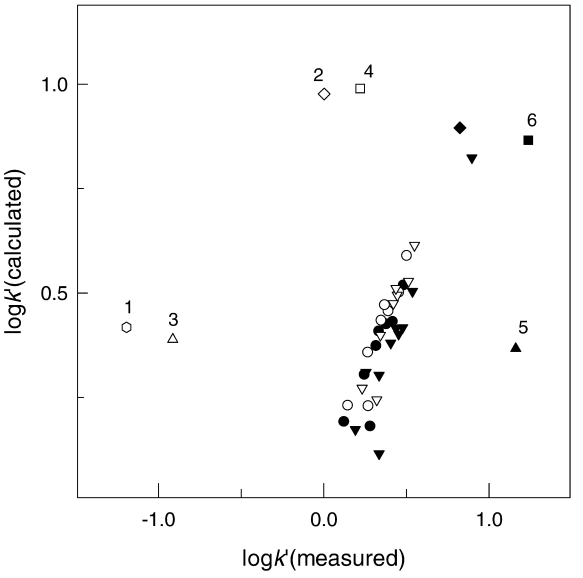


Fig. 5. Comparison of calculated and measured data on retention (log k') of phenols on PhA-silica and Ph-silica at pH 3.54 and 6.00 of acetonitrile–sodium acetate buffer (40:60, v/v). Outliers: 1 and 2 are 2,4-dinitrophenol and pentachlorophenol on Ph-silica at pH 6.00; 3 and 4 are 2,4-dinitrophenol and pentachlorophenol on PhA-silica at pH 6.00 and 5 and 6 are 2,4-dinitrophenol and pentachlorophenol on Ph-silica at pH 3.54.

individual descriptor values were used for determination of LSER coefficients (Table 4). LSER coefficients for PhA-silica and Ph-silica and regression statistics calculated on the base of these 24 analytes are presented in Table 5. The fact that only two coefficients v and r are positive for both phases, indicates that retention of the analytes is governed mainly by hydrophobic and $\pi-\pi$ interactions. The effect of eluent pH on the coefficients is practically negligible, which is in a good accordance with experiment. Comparison of relative coefficients r/v, s/v, a/v and b/v for two phases showed their polarizability (s), hydrogen bond acceptor (b) and donor (a) properties to be close, while the impact of $\pi-\pi$ interactions on retention (r) is practically two-times higher for PhA-silica. A higher degree of polarizability in the case of PhA-silica is natural, because the attachment of the secondary amino group to the phenyl ring contributes additional electronegativity. Selectivity predicted for separation of phenols on PhA-silica by means of LSERs was compared to experimental results (Table 6). The absence of coincidence in predicted and experimental data for 2,4-dinitrophenol and pentachlorophenol (Fig. 5), those are completely or partially dissociated under the chromatographic conditions, is not surprising, as the use of LSERs is valid only for non-charged solutes. For modelling of retention of ionic compounds also one can use a modified LSER model, which was proposed by Bolliet et al. [35]. The correlation between calculated and measured retention of non-dissociated is not strong enough (Table 7). The worst correlation is for PhA-silica at eluent pH 3.54. The possible source of mistake can be in not enough representative set of test solutes for protonated stationary phase or in fundamental inability of the LSER model to describe its properties.

3.5. Separation of phenols

An example of the separation of 10 phenols on PhA-silica under isocratic conditions is presented in Fig. 6a. To show differences in the selectivity of PhA-silica and Ph-silica for phenols the same separation was conducted on Ph-silica (Fig. 6b).

Table 7
Correlation between calculated and measured retention (log k') of phenols on PhA-silica and Ph-silica ($n=8$, $P=0.95$)[a]

Log k'(calculated) $= b_0 + b_1$ log k'(measured)			
Stationary phase and pH of the eluent	b_0	b_1	R
PhA-silica, pH 3.54	0.05 (0.07)[b]	0.96 (0.21)	0.880
PhA-silica, pH 6.00	0.05 (0.06)	1.05 (0.21)	0.935
Ph-silica, pH 3.54	−0.02 (0.06)	0.94 (0.13)	0.936
Ph-silica, pH 6.00	−0.03 (0.08)	1.15 (0.18)	0.934

[a] Eluent: acetonitrile–5 mM sodium acetate buffer (40:60, v/v).
[b] Numbers in parentheses indicate the standard deviation in the coefficient.

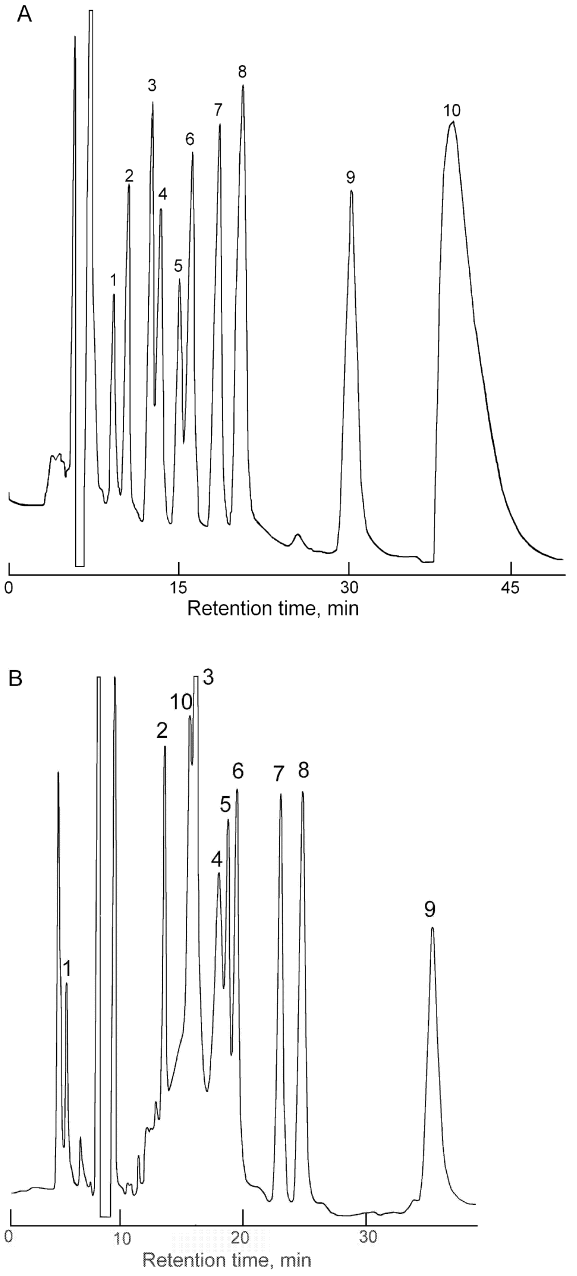


Fig. 6. Separation of a standard mixture of 10 phenols. Column: (a) PhA-silica, 150×4.6 mm with a 40×3 mm pre-column. Flow-rate 0.5 ml/min. (b) Ph-silica, 250×4.6 mm. Flow-rate 0.6 ml/min. Eluent: acetonitrile–5 m*M* sodium acetate buffer (35:65, v/v), pH 4.7. Detection: photometric, λ=280 nm. 1=2,4-Dinitrophenol, 2=phenol, 3=*p*-cresol, 4=*p*-nitrophenol, 5=*p*-chlorophenol, 6=*o*-nitrophenol, 7=3-methyl-3-chlorophenol, 8=2,4-dichlorophenol, 9=2,4,5-trichlorophenol, 10=pentachlorophenol.

4. Conclusions

The new promising stationary phase – 3-phenylaminopropyl silica – was proposed for RP-HPLC. This and a structurally-related phenyl-containing stationary phase differing by the presence of a secondary amino functional group attached to the silica molecule are compared under RP-HPLC conditions for different phenols. The obtained regularities demonstrate a small impact of possible hydrogen-bonding and ion-exchange interactions on the retention of phenol and its chloro-, nitro- and methyl derivatives. The LSER model coefficients were obtained for these two stationary phases and a markable difference was noted only for coefficient *r* which reflects the impact of $\pi-\pi$ interactions on retention. Under optimal conditions the separation of 10 phenols was achieved.

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