

Characterization of phenyl-type HPLC adsorbents

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Abstract

A set of different phenyl-modified HPLC adsorbents were characterized in terms of their surface area, pore volume, and bonded phase volume using low temperature nitrogen adsorption (LTNA). Adsorbents pore volume and interparticle volume were also measured using HPLC. Comparison of the pore volumes assessed with LTNA and HPLC suggests a compact molecular arrangement for all bonded phases studied. Simple and effective method for determination of the exact mass of adsorbent and total surface area in the column is suggested.

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1. Introduction

Stationary phases containing phenyl functionality are gaining popularity as packing materials for reversed-phase HPLC [1–3]. The influence of phenyl functionality of the bonded phase on the retention of model aromatic analytes has been studied on a structure-retention correlative basis [4]. It was shown that while the logarithmic dependencies of homologous series retention on the number of carbon atoms for methanol/water eluents are linear, the same dependencies in acetonitrile/water and tetrahydrofuran/water are non-linear indicating principal differences in the retention mechanism in these systems.

Comparative thermodynamic studies of alkylbenzenes and PAHs' retention on phenyl-modified silicas and conventional C-18-type phases [5] indicate lower overall interaction energies of phenyl phases compared to C-18 modified silicas. However, phenyl phases show greater specificity

towards PAH's indicating additional selectivity due to π – π interactions.

Depending on the model adopted for the description of the HPLC process, analyte retention is proportional to either stationary phase volume (partitioning model) or adsorbent surface area (adsorption model) [6–8]. In both cases proper characterization of packing material geometry is necessary. Usually, pore volume and surface area of base silica could be obtained from the manufacturer; however these parameters for modified silica usually are unavailable. The significance of the pore volume variation between different alkyl-modified silicas was shown in a prior publication [9]. In the same publication the uncertainty in the determination of the surface area of reversed-phase adsorbents was discussed and the use of the base silica surface as a comparable reference was suggested.

In this work we verify an applicability of the same principles outlined in [9] for characterization of several reversed-phase materials with phenyl functionality. For correct thermodynamic interpretation of any retention dependencies or adsorption isotherms the information about the total surface area of adsorbent in the column (or mass of the adsorbent) is necessary. We develop a simple and consistent method for nondestructive experimental determination of the adsorbent mass in the column.

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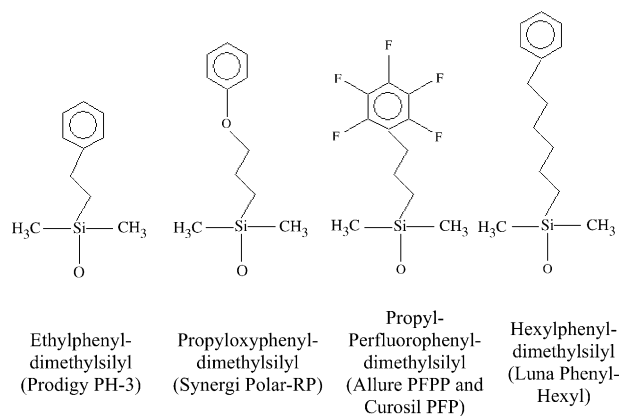


Fig. 1. The structures of the phenyl-bonded ligands.

2. Experimental

2.1. Columns

Four phenyl-type modified silica columns (4.6 mm × 150 mm) were obtained from Phenomenex (Torrance, CA, USA). One perfluorophenyl-dimethylsilyl modified silica column (trade name: Allure PFPP) was donated by Restek (Bellefonte, PA, USA). The structures of the phenyl-type bonded phases are shown in Fig. 1. The average pore diameters and particle sizes of phenyl-type bonded phases supplied by Phenomenex and Restek are shown in Table 1. The bulk unmodified silicas and phenyl-type modified silicas of the identical batches as in the packed columns were also characterized in this study.

2.2. Packing materials

Carbon analysis was performed by Schwarzkopf Micro-analytical Lab (Woodside, NY) using the ASTM method on a Perkin-Elmer 2400 CHN Analyzer using the ASTM method. The carbon percent data were used for the calculation of the bonding density of modified materials. The equation derived by Berendsen and de Galan [10] was used for conversion of experimentally measured carbon content into the bonding density, and calculated values for all studied phenyl-modified adsorbents are shown in Table 1.

2.3. Low-temperature nitrogen adsorption

Bare silica and chemically modified silicas were characterized using the nitrogen adsorption system model 100CX (Omnisorb, NJ, US). The unmodified silicas were degassed under vacuum (4×10^{-5} Torr) at 150 °C for 3 h. The phenyl-type modified silicas were degassed under vacuum without heating. After heating and degassing of the unmodified silicas, the vial was cooled, weighed and placed into the adsorption instrument for analysis. Helium gas was used to calibrate the internal volume of the sample vessel. Static adsorption mode was utilized for all measurements with equilibrium criteria of 0.01% tolerance for nine consequent sampling points. Pore volume was determined from the volume of liquid nitrogen used for the complete filling of all pores (flat region on nitrogen adsorption isotherm at $p/p_s \approx 1$).

2.4. HPLC systems

Two HPLC systems were used: HPLC System I: 1100 HPLC system (Agilent, Palo Alto, CA, USA) equipped with ERMA refractive index detector (ERMA, Kingston, MA, USA); HPLC System II: HP 1050 HPLC system with HP1050 UV detector (Hewlett Packard, New Castle, DE, USA) equipped with PE LC-30 refractive index detector (Perkin-Elmer, Wellesley, MA, USA). The column temperature was kept at 25 °C for both systems. System volume was determined by the elution of 0.1 μL of deuterated acetonitrile in pure acetonitrile in triplicate using RI detection. All eluents were degassed with an inline degasser (Phenomenex, Torrance, CA, USA). Acetonitrile (MeCN), methanol (MeOH) and tetrahydrofuran (THF) were HPLC grade and purchased from Pharmco (Philipsburg, PA, USA). Deuterated MeCN, deuterated MeOH, and deuterated THF were purchased from Sigma Aldrich (St. Louis, Mo, USA). All HPLC experiments were conducted in isocratic mode.

The inter-particle volumes of all studied bonded phases were determined by size-exclusion chromatography (SEC). A series of polystyrene standards with high molecular weights of 97.2, 194, 470, 860, 1840, and 2700 K (Sigma Aldrich, St. Louis, Mo, USA) were prepared in HPLC grade THF (Mallinckrodt, MA, USA). All HPLC data was acquired on Chemstation software v5.0 (Agilent, Palo Alto, CA, USA).

Table 1
Parameters of used packing materials

Packing material	D_p (nm)	S_{BET} (m ² /g)	d_p (μ)	P_c (%)	M (g/mole)	d_b (μmol/m ²)
Prodigy-PH3	9.9	344	5	9.68	163	2.69
Synergi Polar-RP	9.2	381	4	14.42	193	3.63
Curosil PFP	11.6	263	5	10.30	267	3.75
Allure PFPP	6.4	459	5	16.3	267	4.01
Luna Phenyl-Hexyl	11.0	357	5	17.54	219	3.79

D_p is the pore diameter of base silica (provided by manufacturer); S_{BET} is the surface area of base silica (measured in our laboratory); d_p is the average particle diameter; P_c is measured carbon content; M is the molecular weight of attached ligands; d_b is the calculated bonding density.

Table 2
Summary of geometric parameters of bare silica and modified material determined by LTNA

	Silica			Modified adsorbent	
	S (m ² /g)	C	V_p (mL/g)	C	V_p (mL/g)
Prodigy-PH3	344	80	0.97	37	0.69
Synergi Polar-RP	381	77	1.00	32	0.59
Curosil PFP	263	111	0.92	42	0.59
Allure PFPP	459	163	1.10	40	0.52
Luna Phenyl-Hexyl	357	195	1.00	31	0.51

Nitrogen molecular area of 16.2 Å² was used in calculating the BET surface area of the unmodified adsorbent.

3. Result and discussion

3.1. BET surface area and pore volume determination by LTNA

The pore volumes and surface areas of all base silica materials were measured by LTNA, resulting values are shown in Table 2. The adsorbent pore volumes were calculated from the upper plateau of the adsorption isotherm, which corresponds to complete pore filling [11]. The surface area calculation using BET treatment [12] is dependent upon the molecular cross-sectional area occupied by nitrogen gas on the silica surface. For unmodified silica surfaces, 16.2 Å² has been adopted as the nitrogen cross-sectional molecular area for calculation of BET surface area [13]. However, the molecular cross-sectional area occupied by nitrogen molecules on modified silica may be significantly higher than on bare silica due to the weaker interaction of nitrogen molecules with hydrophobic surface [11,14]. BET description of nitrogen adsorption isotherms allows the calculation of the C -constants, which essentially represents the interaction energy between nitrogen and the adsorbent surface.

$$\frac{p/p_s}{n(1-p/p_s)} = \frac{1}{n_m C} + \frac{C-1}{n_m C} \frac{p}{p_s} \quad (1)$$

where p is an equilibrium pressure of adsorbate; p_s is an adsorbate saturation pressure; n is an amount of nitrogen adsorbed on the surface; n_m is the maximum amount of nitrogen which could be adsorbed in dense monomolecular layer; and C is a constant reflecting the adsorption energy. According to Gregg and Sing [11] the relationship between C -constant of BET equation and real interaction energy is only approximate, but it allows for a phenomenological comparison of adsorbent surface energy. C -constant is usually referred as

$$C = \exp\left(\frac{Q_a - Q_L}{RT}\right) \quad (2)$$

where Q_a is the heat of adsorption and Q_L is the heat of condensation.

In the Table 2 C -constants for silica surfaces are shown and C values vary between 80 and 195. Since, the C -constant approximately represents the exponent of the difference between the nitrogen adsorption and condensation energy, we

may expect significant variations for C values when interactions are strong on silica surfaces. Values of C -constant are more consistent for phenyl-modified surfaces, as shown in Table 2, where these values vary between 31 and 37 for phenyl-terminated ligands. For perfluorinated phenyl these values are slightly higher (40–42) due to higher π -electron density of fluorinated benzene rings which increases nitrogen interactions with the surface. Alkyl-modified adsorbents of C-18 type usually show lower C -constant values on the level of 16–20 and the most hydrophobic methyl-terminated surfaces show values on the level of 10–12 [15]. The lower the C -constant value the weaker the interaction of the nitrogen molecules with the surface, which will lead to the increase of its molecular area. Thus, we may expect that on phenyl surface this nitrogen molecular area will be relatively smaller than on C-18-type surface (usually estimated on the level of 21 Å²/molecule), while still higher than on bare silica (16 Å²/molecule).

Due to the significant uncertainty in the nitrogen molecular area on hydrophobic surfaces and also due to the expected significant surface roughness of modified adsorbents all data in this study when necessary were related to the unit of surface of base silica. This approach also allows legitimate comparison between adsorbents with different bonded ligands and bonding density.

By comparison of the pore volume values measured for both modified and unmodified adsorbents there is approximately a two times decrease of the pore volume after modification with the phenyl ligands. These values, although, are related to one gram of measured adsorbent and could not be directly compared. Accurate comparison between pore volumes of unmodified and modified material could only be made if both are related to one gram of unmodified bare silica, as it was discussed in previous paper [9]. Corresponding correction factor for the increase of the adsorbent weight after modification was introduced as:

$$f_{\text{corr}} = \frac{1}{1 + (d_{\text{bond}} S_{\text{SiO}_2} M_{\text{ligand}}) 10^{-6}} \quad (3)$$

where d_{bond} is bonding density of the ligand ($\mu\text{mol}/\text{m}^2$), S_{SiO_2} is the surface area of the unmodified silica (m^2/g) and M_{ligand} is the molecular weight of the bonded ligand (g/mole). The product of these three terms represents the mass of the bonded ligands in 1 g of native silica. In essence, f_{corr} is the ratio between 1 g of native silica and the mass of the same silica plus ligands bonded onto it. Surface areas of unmodified (native) silicas are shown in Table 1. All other geometric parameters required to determine f_{corr} and calculated correction factors for phenyl-type modified silica are listed in Tables 1 and 3.

Pore volume corrected for the adsorbent weight increase after bonding essentially represents the actual pore volume of modified adsorbent related to one gram of bare silica, and thus could be compared to the original specific pore volume of silica. The difference between these values represents the volume of bonded phase on one gram of silica. Since the

Table 3
LTNA effective molecular volumes of the phenyl-type bonded ligands

1	2	3	4	5	6	7	8
Bonded phase	V_{pore} (mL/g _{modified silica})	f correction factor	V_{pore} corrected (mL/g _{bare silica})	V_{pore} base silica	$V_{\text{bonded layer}}$ (mL/g _{bare silica})	$V_{\text{effective}}$ molecular volume (\AA^3 /molecule)	V_{ACD} molecular volume (\AA^3 /molecule)
Prodigy PH-3	0.687	0.869	0.791	0.97	0.179	321	314
Synergi Polar-RP	0.592	0.789	0.750	1.00	0.25	301	326
Curosil PFP	0.590	0.792	0.745	0.92	0.175	295	312
Allure PFPP	0.515	0.671	0.768	1.10	0.332	300	312
Luna Phenyl-Hexyl	0.508	0.772	0.658	1.00	0.342	420	410

bonding density (d_b) and silica surface are known (Table 1) it is possible to calculate effective molecular volume of bonded ligands as

$$V_{\text{ligand}} = \frac{V_{\text{bonded layer}}}{S d_b N_A} \quad (4)$$

where $V_{\text{bonded layer}}$ is the bonded layer volume, S is the silica surface area, d_b is the bonding density and N_A is the Avogadro number.

ACD software [16] was used to evaluate the theoretical liquid phase densities and corresponding molecular volumes of the phenyl bonded chains, column 8, Table 3. Due to the inability of the software used to estimate the theoretical molecular volume of dimethylsilyl base, the silicon atom in each bonded ligand was substituted with a carbon atom in the molecular volume assessment. The effective molecular volumes using parameters determined by LTNA are shown in column 7, Table 3. Molecular volumes calculated by ACD software are based on the density of corresponding liquids, and essentially represent the most compact volume occupied by the molecule in incompressible liquid. As it could be seen from Table 3 the molecular volumes obtained from LTNA and ACD prediction values are quite comparable. This implies that molecular arrangement of the phenyl bonded chains under LTNA condition in vacuum is as compact as in the liquid phase. However, the molecular arrangement of these bonded ligands in HPLC conditions where they are exposed to organic eluent at room temperature may be different from that in LTNA environment. Thus, it would be important to compare the pore volumes of modified adsorbents measured by LTNA with those measured by HPLC.

Porosity in HPLC column consists of two parts: the packing material pore volume (V_p) and interparticle volume (V_{ip}). The sum of these values constitutes the total volume of the liquid phase in the column or the void volume (V_0). The V_0 can be accurately measured by HPLC using minor disturbance and deuterated component methods [9]. V_{ip} can be determined as the exclusion volume using size-exclusion chromatography [9]. The difference in volumes between void and inter-particle yields the column pore volume determined in HPLC eluent conditions.

3.2. Void volume determination

The void volumes of HPLC columns packed with the different reversed-phase adsorbents were measured with minor disturbance method (MDM) [17] and retention of deuterated eluent components for acetonitrile/water, methanol/water and tetrahydrofuran/water combinations.

The void volumes of all phenyl-type bonded phases were determined in two binary systems: acetonitrile/water and methanol/water using MDM. V_0 for Curosil PFP and Luna Phenyl-Hexyl modified adsorbents were also determined by MDM using a tetrahydrofuran/water eluent system. In addition, void volumes of all phenyl-type bonded phases were also determined by the retention of deuterated acetonitrile eluted in pure acetonitrile, deuterated methanol eluted in pure methanol and deuterated tetrahydrofuran eluted in pure tetrahydrofuran respectively. Experimental data (retention volumes) for V_0 determination by minor disturbance method for different binary eluent combinations are shown in Appendix A Tables A.1–A.3. V_0 of the four phenyl-type bonded stationary phases determined using both methods are summarized in Table 4.

Representative dependencies of minor disturbance retention volumes of phenyl-type bonded phases on MeCN:H₂O, MeOH:H₂O and THF:H₂O binary eluent systems are illustrated in Fig. 2. The void volumes of each phenyl-type column determined using minor disturbance and deuterated components are very consistent. Overall RSDs for each column is less than 2%. This implies that the conformation of the bonded phenyl ligands does not alter as a function of solvent hydrophobicity and protic nature.

3.3. Exclusion volume and packing density determination

The inter-particle volume (V_{ip}) is the volume between packed adsorbent particles within an analytical RP-HPLC column and it could be measured as the retention volume for the molecules completely excluded from the porous space during the elution process. This measurement requires the use of relatively large polymer molecules, which have their own significant volume, thus the exclusion volume measured with those molecules will have additional exclusion from the

Table 4
Void volume values measured with MeCN–water, MeOH–water, THF–water, minor disturbance method and deuterated components

V_0 (mL)	Minor disturbance			Deuterated components			Avg	%RSD
	MeCN	MeOH	THF	Deuterated MeCN	Deuterated MeOH	Deuterated THF		
Columns								
Prodigy PH-3	1.881	1.829	na	1.850	1.840	1.829	1.846	1.2
Synergi Polar-RP	1.755	1.708	na	1.726	1.746	1.714	1.730	1.2
Curosil PFP	1.768	1.734	1.763	1.744	1.750	1.744	1.751	0.7
Luna Phenyl-Hexyl	1.693	1.631	1.671	1.676	1.660	1.631	1.660	1.5

na, the minor disturbance analysis was not performed on this adsorbent.

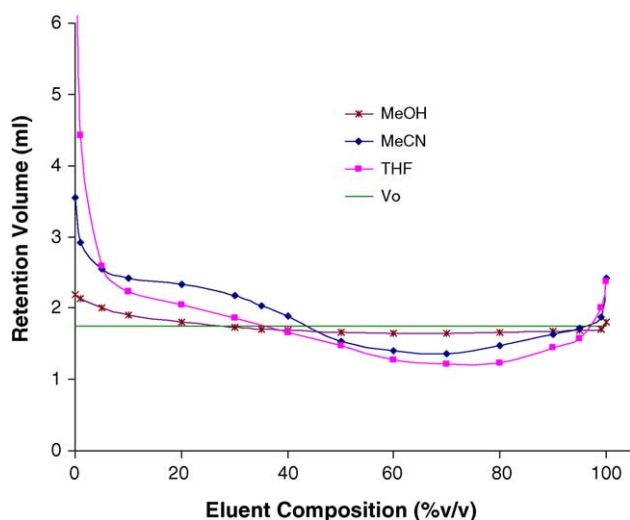


Fig. 2. Dependencies of the minor disturbance peaks for MeCN:H₂O; MeOH/water; and THF/water systems on a Curosil PFP column.

adsorbent particle surface based on the value of their gyration radius. In the first approximation, the gyration radius of the polymer molecules in solution is proportional to the cubic root of its molecular weight. For completely excluded molecules, a linear dependence between retention volume and the cubic root of the molecular mass of the respective polymer standard was shown [9]. The inter-particle volume of the column is determined by extrapolation of the exclusion branch of the SEC curve to zero mass point on the x -axis (retention volume).

The total exclusion volumes of all phenyl-type bonded phases were determined by eluting a series of polystyrene standards with molecular masses of 97.2, 194, 470, 860,

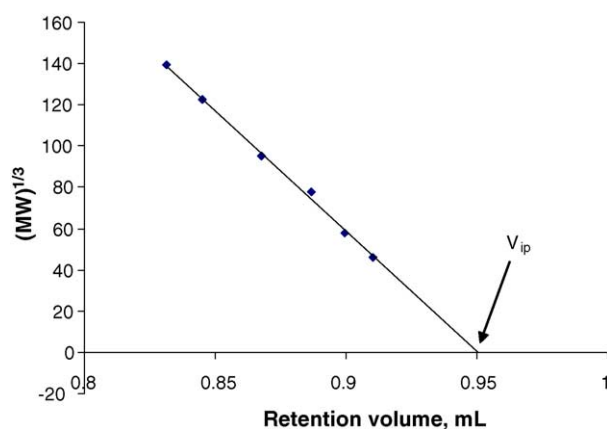


Fig. 3. Decrease of retention volume due to the molecular size of polystyrene standards on a Synergi Polar-RP column.

1840, and 2700 K in THF on each phenyl-type bonded phase. Experimental data (retention volumes) for V_{ip} determination by SEC using THF mobile phase are shown in Appendix Table A.4. The extrapolation of the column inter-particle volume from the plot of cubic root of polymer standard molecular weights versus retention volumes is shown in Fig. 3. The inter-particle volumes (V_{ip}) of all phenyl-type bonded phases were summarized in column 3, Table 5.

3.4. Comparison of pore volumes obtained from LTNA versus HPLC

Adsorbent pore volume significantly decreases as a result of modification of silica surface with organic moieties. In Section 3.1 we demonstrated that the volume of bonded phase in

Table 5
Column parameters

1	2	3	4	5	6	7
Method	HPLC	SEC	Weight	Calculated	LTNA	% Difference
Parameter	V_0 (mL/column)	V_{ip} (mL/column)	m_{ads} (g/column)	V_{pore} (mL/g)	V_{pore} (mL/g)	
Column						
Prodigy PH-3	1.846	0.962	1.27	0.696	0.687	1.3
Synergi Polar-RP	1.730	0.950	1.29	0.604	0.592	2.0
Curosil PFP	1.751	0.939	1.40	0.580	0.590	1.7
Allure PFPP	1.65	0.930	1.35	0.530	0.515	2.9
Luna Phenyl-Hexyl	1.660	0.923	1.45	0.508	0.508	<0.1

V_0 , void volume (average values measured using MDM and isotopic retention); V_{ip} , interparticle volume (SEC measured); V_{pore} , pore volume of modified adsorbent determined by LTNA; m_{ads} , mass of adsorbent measured by weighing of unpacked material.

vacuum environment corresponds to the dense arrangement of bonded ligands. The difference between the column void volume and interparticle volume essentially represents the pore volume of the adsorbent in the column. If the mass of adsorbent in the column is known the specific adsorbent pore volume assessed under HPLC conditions could be calculated.

Chemical modification of the silica surface would only affect the pore volume; it would not noticeably alter the particle diameter. Hence, comparison of pore volumes determined from LTNA and HPLC is justifiable. If the conformation of the phenyl bonded phases in LTNA and HPLC conditions were similar, the pore volumes determined by both techniques should be quite comparable. The specific pore volume of modified adsorbent ($V_{p\text{ modified}}$) can be determined on the basis of HPLC data using the following equation:

$$\frac{V_0 - V_{ip}}{m_{\text{ads}}} = V_{p\text{ modified}} \quad (5)$$

where V_0 is the column void volume; V_{ip} is the inter-particle volume of the column, m_{ads} is the mass of the modified silica.

All columns used in this study were unpacked and adsorbents dried at 80 °C until constant weights were achieved. The V_0 , V_{ip} , and m_{ads} values are summarized in Table 5 along with the values of specific pore volumes calculated using Eq. (5) and measured by LTNA. The average deviation between the calculated and measured pore volume values (columns 5 and 6 in Table 5, respectively) is less than 3%, which essentially allows concluding that molecular arrangement in the bonded layer exposed to HPLC mobile phase is similar to that in vacuum.

Correlation of the pore volume measured by LTNA and by HPLC suggests a convenient method for the determination of the exact mass of the adsorbent in the HPLC column without unpacking. For the calculation of adsorbent mass by Eq. (5) one would need to determine the column void volume, interparticle volume in the column and specific pore volume of modified adsorbent. Column void volume could be measured by the retention of deuterated acetonitrile eluted from pure acetonitrile as an eluent and interparticle volume could be measured by the retention of a simple set of polystyrene standards from THF.

The situation with the specific pore volume of modified adsorbent is more complex. Column manufacturers usually

can provide pore volume and adsorbent surface area for base silica and not for modified adsorbents. As we demonstrated above, molecular volumes of bonded ligands on the surface corresponds to their normal molecular volumes in the liquid state and could be estimated either from density values or calculated using molecular modeling software, such as ACD. This allows for theoretical calculation of the pore volume of the modified adsorbent if bonding density is known. The decrease of the pore volume (ΔV , mL/g) due to chemical modification will be equal to

$$\Delta V = S \cdot d_b \cdot V_m \cdot N_A \quad (6)$$

where S is the base silica surface area (m^2/g), d_b is the bonding density ($\mu\text{mole}/\text{m}^2$), V_m is the molecular volume of bonded ligands ($\text{\AA}^3/\text{molecule}$), N_A is the Avogadro number. Specific pore volume of the modified adsorbent related to one gram of base silica will be

$$V_{p\text{ modified SiO}_2} = V_p - \Delta V \quad (7)$$

For the purpose of determination of the adsorbent mass in the column we will need the specific pore volume related to one gram of modified adsorbent, which could be calculated using the correction factor given in Eq. (3). The resulting expression (without units' conversion) will be

$$V_{p\text{ modified}} = (V_p - Sd_b V_m N_A)(1 + Sd_b MW) \quad (8)$$

where MW is the molecular weight of the bonded ligand. Comparison of measured (by LTNA) and calculated (using Eq. (8)) adsorbent pore volumes are shown in columns 4 and 3, respectively in Table 6. Average deviation between measured and calculated pore volume values is 1.6%.

Also, the calculated (using Eq. (5)) and measured adsorbent mass are shown in columns 6 and 7, respectively in Table 6. In the calculation for adsorbent mass in the column the experimentally determined V_0 and V_{ip} values were used along with specific pore volume of modified adsorbent (using Eq. (8)). Average deviation between measured and calculated values is 2.5% with maximum deviation of 5%. Given the complexity of measurements involved and assumptions made these results for the adsorbent mass are surprisingly close, which allows us to suggest it as a good alternative to the column unpacking.

Table 6
Calculation of the adsorbent mass in the column

1	2	3	4	6	7	8
Bonded phase	Bonded phase volume (calculated) (mL/g _{SiO₂})	Adsorbent pore volume (calculated-Eq. (8)) (mL/g _{modified})	Adsorbent pore volume (measured LTNA) (mL/g _{modified})	Adsorbent mass (calculated Eq. (5)) (g/column)	Adsorbent mass (measured) (g/column)	% Difference
Prodigy PH-3	0.175	0.691	0.687	1.28	1.27	0.8
Synergi Polar-RP	0.272	0.575	0.592	1.35	1.29	4.7
Curosil PFP	0.185	0.582	0.590	1.39	1.40	0.7
Allure PFPP	0.346	0.506	0.515	1.42	1.35	5.1
Luna Phenyl-Hexyl	0.334	0.514	0.508	1.43	1.45	1.4

4. Conclusions

Characterization of the geometric parameters of phenyl-type HPLC stationary phases show their similarity in terms of dense molecular arrangement of bonded ligands on the silica surface with previously characterized alkyl-type phases [9].

The method for the determination of the exact mass of adsorbent in the column is suggested. It is advisable to relate any thermodynamic parameters measured in HPLC to the unit of the adsorbent surface, since the HPLC retention is essentially proportional to the adsorbent surface area. The

comparison of results obtained on columns with different bonding densities and even modified with different ligands could be significantly more consistent if results are related to the unit of the silica surface. The reported procedure makes this approach feasible.

Acknowledgements

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Appendix A

See Tables A.1–A.4.

Table A.1

Minor disturbance peak retention volumes of phenyl-type bonded phases in acetonitrile-water binary system

MeCN concentration (% , v/v)	System volume corrected retention volumes			
	Prodigy PH-3	Synergi Polar-RP	Curosil PFP	Luna Phenyl-Hexyl
100	4.838	4.698	2.357	2.723
99	2.545	2.162	1.853	1.944
95	2.049	1.658	1.825	1.587
90	1.684	1.525	1.555	1.491
80	1.492	1.366	1.397	1.348
70	1.365	1.273	1.297	1.257
60	1.358	1.278	1.284	1.272
50	1.497	1.380	1.422	1.386
40	1.746	1.606	1.699	1.608
35	1.891	1.735	1.831	1.730
30	2.029	1.874	1.953	1.838
20	2.256	2.116	2.154	2.042
10	2.609	2.555	2.344	2.243
5	2.650	2.708	2.677	2.426
1	2.770	2.738	3.027	3.050
0	3.165	3.502	3.613	3.776

Table A.2

Minor disturbance peak retention volumes of phenyl-type bonded phases in methanol-water binary system

MeOH concentration (% , v/v)	System volume corrected retention volumes			
	Prodigy PH-3	Synergi Polar-RP	Curosil PFP	Luna Phenyl-Hexyl
100	2.003	1.800	1.803	1.753
99	1.902	1.739	1.701	1.719
95	1.801	1.677	1.685	1.610
90	1.779	1.659	1.673	1.589
80	1.745	1.629	1.657	1.561
70	1.727	1.611	1.647	1.547
60	1.733	1.614	1.646	1.543
50	1.757	1.632	1.662	1.558
40	1.799	1.668	1.690	1.582
35	1.832	1.697	1.711	1.602
30	1.862	1.722	1.737	1.619
20	1.917	1.789	1.802	1.675
10	1.973	1.872	1.908	1.776
5	2.021	1.935	2.005	1.876
1	2.116	2.028	2.132	2.018
0	2.195	2.092	2.186	2.102

Table A.3
Minor disturbance peak retention volumes of phenyl-type bonded phases in tetrahydrofuran-water binary system

THF concentration (% v/v)	System volume corrected retention volumes	
	Curosil PFP	Luna Phenyl-Hexyl
100	2.371	1.556
99	1.998	1.775
95	1.582	1.459
90	1.440	1.341
80	1.232	1.232
70	1.212	1.212
60	1.274	1.274
50	1.474	1.389
40	1.666	1.550
30	1.858	1.738
20	2.048	1.879
10	2.236	2.013
5	2.598	2.334
1	4.423	4.101
0	10.039	13.381

Table A.4
Retention volumes of polystyrene standards determined by size-exclusion chromatography*

Polystyrene standard (MW)	System volume corrected retention volumes using size-exclusion chromatography			
	Prodigy PH-3	Synergi Polar-RP	Curosil PFP	Luna Phenyl-Hexyl
97200	1.879	1.850	1.831	1.791
194000	1.861	1.829	1.813	1.772
470000	1.839	1.802	1.790	1.746
860000	1.805	1.763	1.756	1.710
1840000	1.763	1.718	1.718	1.663
2700000	1.734	1.692	1.684	1.631

* Flow rate 0.5 mL/min.

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