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## Geometry of chemically modified silica

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### Abstract

The effect of alkyl chain length on adsorbent pore volume and void volume of HPLC columns is described. The results provide evidence that alkyl chains attached on silica surface are densely packed. A correlation of a decrease of pore volume with an increase of the alkyl modifier chain length was found. Effective molecular volume of bonded chains was found to be similar to the molecular volume of corresponding liquid alkanes. An absence of noticeable penetration of acetonitrile, methanol, or tetrahydrofuran molecules between bonded chains at any water–organic eluent composition was found. © 2001 Elsevier Science B.V. All rights reserved.

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### 1. Introduction

The adsorbent surface area, bonded phase chemistry and bonding density are among the major factors affecting the reversed-phase separation [1]. The majority of adsorbents for reversed-phase high-performance liquid chromatography (HPLC) are mesoporous silica gels with chemically modified hydrophobic surfaces. Most HPLC silicas have pore diameters between 60 and 150 Å, surface areas between 120 to 450 m<sup>2</sup>/g and pore volumes within the range of 0.5 to 1.2 ml/g. Most of the bonded phases used are of the C<sub>18</sub> type and the average bonding density for monomeric phases is above 2.5 μmol/m<sup>2</sup>.

The binding of significant amounts of large alkyl

groups, such as C<sub>18</sub>, on the silica surface will alter the adsorbent geometry and will influence the mechanism of separation [2]. Bonded alkyl chains also occupy volume inside the pore space and are expected to decrease original silica pore volume. A corresponding decrease of the adsorbent surface area and average pore diameter is also expected. However, these effects and their consequences on HPLC separations have not been fully addressed in the literature and the interpretation of the experimental results are controversial [2–9].

Low-temperature nitrogen adsorption (LTNA) is commonly used for geometric characterization of HPLC adsorbents. Its applicability to characterization of rigid porous materials has been studied for many years and is well-established [10,11]. There is, however, significant doubt regarding LTNA results obtained for adsorbents with various surface chemistries [12,13]. It has been shown [10,12] that nitrogen molecular area, the primary constant used for surface area calculations from LTNA data, is significantly

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greater for hydrophobic surfaces than it is for the classical polar silica gel ( $16.4 \text{ \AA}^2$ ).

Bass et al. [3] studied the effect of surface modification on adsorbent geometry for four different silica gels. Their results clearly show a decrease of pore volume, surface area, and mean pore diameter with an increase of the chain length of the bonded alkyl moiety. Other investigators [2,5,7] reported the same effect of decreasing geometric parameters with increasing length of bonded chain. However, Jaroniec and co-workers [8,9] did not detect a significant decrease of surface area and pore volume after chemical modification of the silica surface.

If it is assumed that bonded *n*-octadecyl chains occupy approximately the same molecular volume when bound to the silica surface as they do in the liquid phase ( $\sim 600 \text{ \AA}^3/\text{molecule}$  or  $361 \text{ ml/mol}$ ), then it is possible to calculate a theoretical decrease of the pore volume after surface modification. For example, for an adsorbent with a surface area of  $300 \text{ m}^2/\text{g}$ , bonded with octadecylchlorosilane with bonding density of  $3 \text{ \mu mol/m}^2$ , a pore volume decrease of approximately  $0.33 \text{ ml/g}$  is expected. If the original silica had  $1 \text{ ml/g}$  original pore volume, the decrease would be about 33%, a very significant decrease.

Experimental data from Refs. [5,7,8] allow calculation of the decrease of the adsorbent pore volume (Fig. 1) with an increase in the alkyl chain length of the bonded ligands. The magnitude of the decrease of the adsorbent pore volume of different silica gels is consistent from three different research groups. The average slope for all three dependencies is  $18 \text{ \mu l/g}$  per  $\text{CH}_2$  group and the standard deviation is less than 15%.

It is possible that the molecular arrangement of the organic bonded layer exposed to the HPLC eluent at ambient temperature is significantly different from that in vacuum at liquid nitrogen temperature. This can be evaluated by comparing the pore volume measured by LTNA and the pore volume measured by HPLC. The pore volume under HPLC conditions is determined by obtaining a correctly measured column void volume,  $V_0$ , exclusion volume,  $V_{\text{ex}}$ , (interparticle volume) and actual mass of adsorbent in the column,  $m_a$ .

The geometric parameters of 10 silica-based ad-

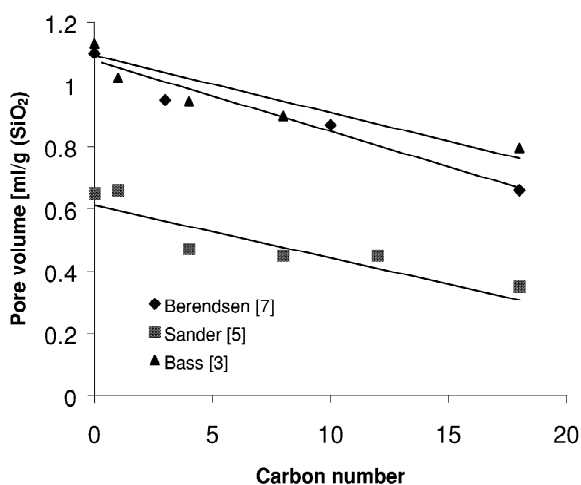


Fig. 1. Decrease of the adsorbent pore volume with increase of the chain length of the bonded ligands. Data calculated from Berendsen et al. [7], Sander et al. [5], and Bass et al. [3]. All data were corrected for the mass of silica.

sorbents modified with ligands of different chain lengths as well as original silica were determined by LTNA. The void volume of HPLC columns packed with different reversed-phase adsorbents was measured with three different methods using various types of solvents and eluent compositions. Adsorbent pore volume calculated as the difference between the column void volume and exclusion volume was compared with the adsorbent pore volume measured under LTNA conditions.

## 2. Experimental

### 2.1. Adsorbents and columns

High-purity porous silica was used in this study. This silica was chemically modified with alkyldimethylchlorosilanes of different alkyl chain lengths including  $C_1$ – $C_6$ ,  $C_8$ ,  $C_{10}$ ,  $C_{12}$  and  $C_{18}$ . The alkylsilylation procedure is described elsewhere [14]. Adsorbents were packed into  $150 \times 4.6 \text{ mm}$  stainless steel columns using the slurry packing procedure. Two sets of columns were studied. The first set includes all adsorbents (10 columns), and the second set which was packed separately (2 months later)

Table 1  
Geometric parameters of bare porous silica and alkylsililated gels by LTNA

1: Adsorbent	2: BET surface area (m <sup>2</sup> /g)	3: Total pore volume (ml/g)	4: Mean pore diameter (Å)	5: “Carbon” Load (C, %, w/w)	6: Bonding density (μmol/m <sup>2</sup> )
Silica	374	0.965	97	0	0
C <sub>1</sub>	292	0.804	88.6	5.03	4.16
C <sub>2</sub>	301	0.804	88.2	6.01	3.76
C <sub>3</sub>	295	0.781	87.1	6.73	3.38
C <sub>4</sub>	299	0.778	86.4	7.84	3.33
C <sub>5</sub>	288	0.746	84.8	8.3	3.03
C <sub>6</sub>	288	0.736	83	9.26	2.99
C <sub>8</sub>	287	0.726	81	10.4	2.72
C <sub>10</sub>	264	0.687	80	11.1	2.44
C <sub>12</sub>	236	0.623	78	13.4	2.61
C <sub>18</sub>	182	0.531	79	17.4	2.51

included columns packed with C<sub>1</sub>, C<sub>2</sub>, C<sub>4</sub>, C<sub>8</sub> and C<sub>18</sub> modified adsorbents.

## 2.2. Low-temperature nitrogen adsorption

Bare silica and chemically modified samples were characterized using the ASAP Model 2010 (Micromeritics, Norcross, GA, USA) LTNA instrument. Adsorbents were degassed under vacuum (10<sup>-5</sup> Torr; 1 Torr = 133.322 Pa) at 350°C (for bare silica)

and at 150°C for chemically modified silica for 12 h in the instrument vial. After cooling, the vial was weighed and placed into the adsorption instrument. A static adsorption mode was used and included full equilibration after each adsorbate load. The instrument temperature (manifold) was 28°C. Geometric parameters including surface area, pore volume, and pore size distribution for all adsorbents measured with LTNA are shown in Table 1. Representative adsorption–desorption isotherms for original silica and C<sub>18</sub> modified adsorbent are shown in Fig. 2.

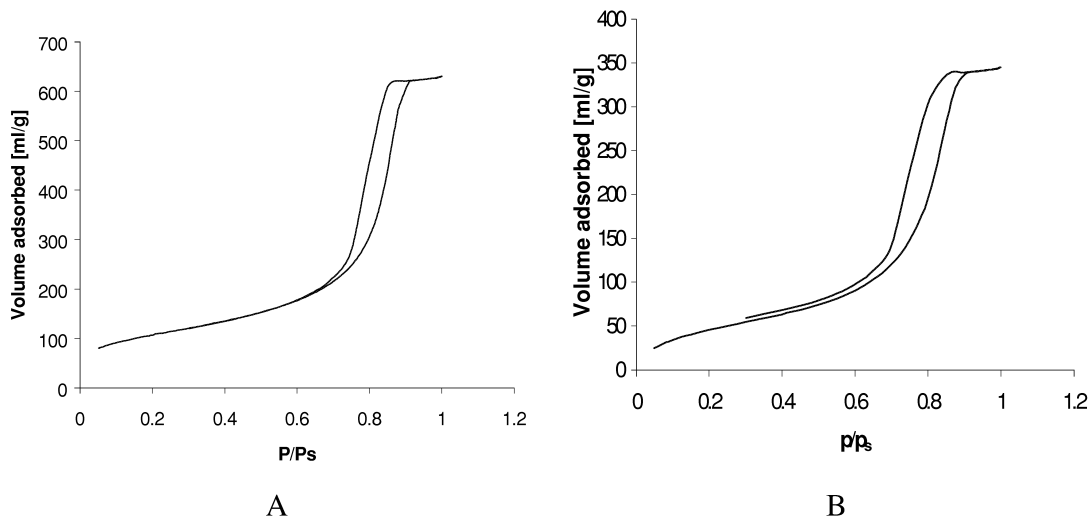


Fig. 2. Nitrogen adsorption isotherms on bare silica (A) and on the same silica modified with octadecylsilane (B).

Adsorbent pore volume was calculated from the upper plateau of the adsorption isotherm, which corresponds to complete pore filling [10]. Surface area values were calculated using the BET method [11]. The carbon load was derived from elemental analysis of the chemically bonded phases. The weight percent of carbon on chemically modified silicas was measured according to the procedure described by Berendsen and de Galan [15]. The bonding densities of the chemically modified silicas were calculated from the weight percent of carbon and the specific surface area of bare silica [15].

### 2.3. HPLC systems

Void volume was determined on two different HPLC systems. System I: HP Model 1050 pump and autosampler (Hewlett-Packard, Little Falls, MD, USA) equipped with a refractive index detector ERC-7510 (Erma, Kingston, MA, USA). System II: PE Model 410 pump (Perkin-Elmer, Norwalk, CT, USA); HP Model 1050 autosampler (Hewlett-Packard) and Model 410 refractive index (RI) detector (Waters, Milford, MA, USA). The column temperature was set at 25°C and controlled by a circulating water-bath (Brinkman Model RC6; Lauda, Lauda-Königshofen, Germany). All eluents were degassed with a degasser unit (Phenomenex, Torrance, CA, USA).

Gel permeation chromatography (GPC) experiments for the determination of the exclusion volume were performed on the third system, consisting of the PE-410 pump, ISS-100 autosampler (Perkin-Elmer) and Model 785A variable-wavelength UV detector (Applied Biosystems, Sunnyvale, CA, USA). A series of high-molecular-mass polystyrene standards of low polydispersity (Polymer Labs., Church Stretton, UK) were dissolved in HPLC-grade tetrahydrofuran (THF) (Sigma, Allentown, PA, USA).

All HPLC systems were equipped with a Turbochrom-4 data acquisition system (Perkin-Elmer).

Extracolumn volumes of all systems were determined by direct connection of column inlet and outlet capillaries. The average retention volume of a 1  $\mu$ l injection of 100 ppm benzene solution at five different flow-rates was used. Geometric parameters

of the HPLC columns were studied using a GPC method (for determination of interparticle volume) and the minor disturbance method (for determination of the column void volume). The minor disturbance method for column void volume determination was described in prior work [16]. The void volume was determined using three different eluent systems: acetonitrile (MeCN)–water, methanol (MeOH)–water, and THF–water at 25°C. The consistency of the void volume measurement was checked with three different methods: minor disturbance; injection of isotopically labeled components [17] and pycnometry [18]. After completion of all experiments, the second set of columns were purged with acetonitrile and unpacked; the adsorbent was dried at 85°C under slow nitrogen flow followed by vacuum until a constant mass was obtained.

All solvents and reagents used were high-purity HPLC-grade (Sigma). Experimental values for the retention of acetonitrile–water minor disturbance peaks were corrected for extracolumn volume for the first and second column sets (see Appendix, Tables A.1 and A.2). Corresponding values for minor disturbance peaks for methanol–water for the first and second column set are shown in Appendix Tables A.3 and A.4, and values for THF–water for the second set of columns are shown in Appendix Table A.5. Representative dependencies of the minor disturbance peaks on the eluent composition for different eluent types on  $C_1$  and  $C_{18}$  columns are shown in Fig. 3.

## 3. Results and discussion

### 3.1. Molecular volume of bonded alkyl ligands

Fig. 1 and Table 1 show that the adsorbent pore volume is dependent on chemical modification of the silica surface, decreasing with increasing length of the bonded alkyl chains. The LTNA measurements gave a specific pore volume relative to the one gram of the sample used as shown in Table 2, column 2. The specific pore volumes for the modified adsorbents are denoted as  $V_p$ . Valid comparisons of the pore volume change due to the surface modification can be made if the measured values are related to the

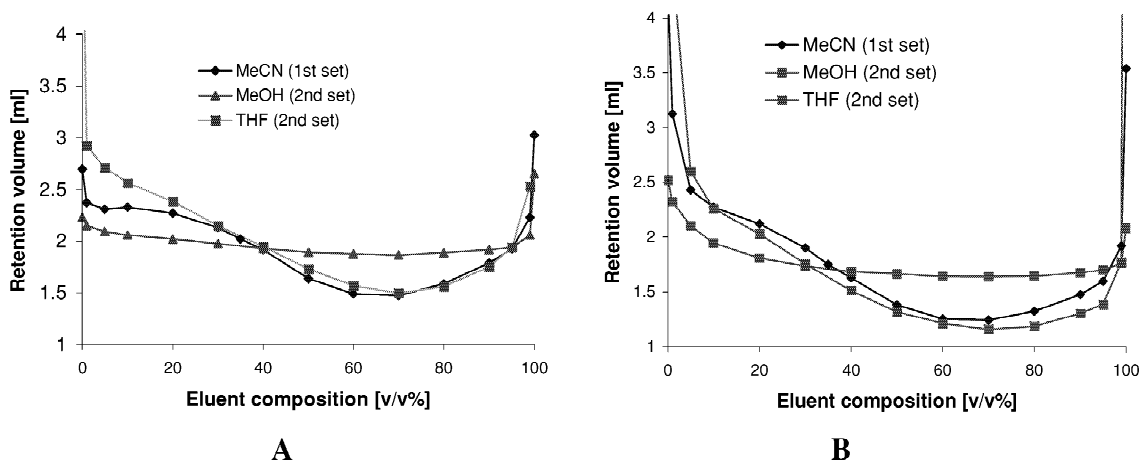


Fig. 3. Representative dependencies of the minor disturbance peaks for different eluent types on  $C_1$  column (A) and on  $C_{18}$  column (B).

same amount of original silica (1 g). These corrected values are denoted as  $V_p^{Si}$  and are also shown in Table 2 (column 3). The correction factor for each modified silica was calculated from the experimental value of the bare silica surface area, bonding density, and type of attached ligands. The product of bonding density ( $d_{bond}$ ,  $\mu\text{mol}/\text{m}^2$ ), specific surface area of silica ( $S_{\text{SiO}_2}$ ,  $\text{m}^2/\text{g}$ ), and molecular mass of bonded ligands ( $M_{r, \text{ligand}}$ ,  $\text{g}/\text{mol}$ ) represents the mass of the

bonded layer on 1 g of bare silica. The correction factor for the calculation of the amount of bare silica in 1 g of modified adsorbent is:

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

The difference between the specific pore volume of bare silica ( $V_{\text{SiO}_2}$ ) and the specific pore volume of

Table 2  
Corrected pore volume and the volume of bonded phase

1: Carbon number	2: Measured pore volume <sup>a</sup> , $V_p$ (ml/g)	3: Corrected pore volume <sup>b</sup> , $V_p^{Si}$ (ml/g) <sub>(SiO<sub>2</sub>)</sub>	4: Bonded layer volume (ml/g) <sub>(SiO<sub>2</sub>)</sub>	5: Bonded layer volume ( $\mu\text{l}/\text{m}^2$ )	6: Effective molecular volume of bonded chains (l/mol)	7: Effective molecular volume of bonded chains ( $\text{\AA}^3/\text{molecule}$ )
0	0.965	0.965	0			
1	0.804	0.894	0.071	0.189	0.045	75
2	0.804	0.901	0.064	0.172	0.046	76
3	0.781	0.880	0.085	0.227	0.067	112
4	0.778	0.889	0.076	0.204	0.061	102
5	0.746	0.854	0.111	0.297	0.098	163
6	0.736	0.853	0.112	0.299	0.100	166
8	0.727	0.853	0.112	0.300	0.110	183
10	0.687	0.811	0.154	0.413	0.169	281
12	0.623	0.760	0.205	0.549	0.210	350
18	0.531	0.685	0.280	0.748	0.298	494

<sup>a</sup> Adsorbent pore volume measured with LTNA as a volume of liquid nitrogen at complete pore filling point (upper plateau in Fig. 2). Expressed in ml/g of modified adsorbent.

<sup>b</sup> Same pore volume as in column 2 recalculated relative to 1 g of base silica.

modified adsorbent corrected to the bare silica mass ( $V_p^{Si}$ ) represents the specific bonded layer volume ( $V_{b.l.}^s$ ). Values are shown in Table 2, column 4. The volume of the bonded layer per unit area of original silica is calculated by dividing the specific bonded layer volume by the specific surface area of bare silica, as shown in Table 2, column 5:

$$V_{b.l.}^a = \frac{V_{b.l.}^s}{S_{SiO_2}} \quad (2)$$

In the above Eq. (2)  $V_{b.l.}^a$  is surface specific volume of the bonded layer,  $V_{b.l.}^s$  is the specific bonded layer volume, and  $S_{SiO_2}$  is a specific surface area of original silica. The bonded layer consists of anchored alkyl moieties. Since the average bonding density is known (Table 1, column 6) it is possible to calculate the effective molecular volume of the bonded chains. This is calculated by dividing bonded layer volume per unit area ( $V_{b.l.}^a$ ) (Table 2, column 5) by its corresponding bonding density value,  $d_b$  (Table 1, column 6). These results are shown in Table 2, columns 6 and 7 in different units.

It is interesting to compare these values with the standard molecular volumes of corresponding liquid  $n$ -alkanes. Each bonded alkylsilane ligand consists of a silicon atom, two side methyl groups, and a corresponding alkyl chain. For example, ligands in the bonded phase denoted as  $C_4$  contain a total of six carbon atoms (butyl chain and two side  $CH_3$  groups).

Fig. 4 shows the close correlation of effective

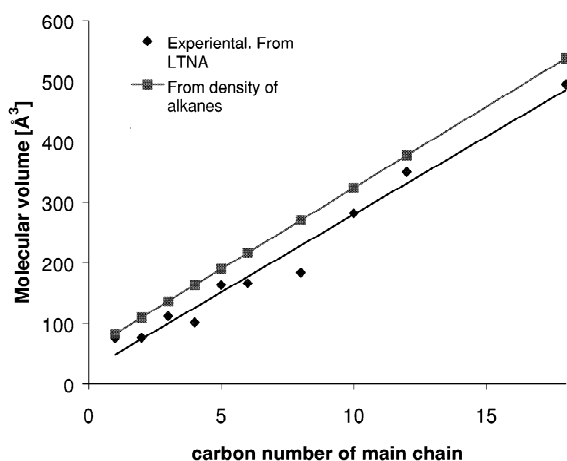


Fig. 4. Comparison of the molecular volumes of bonded ligands with that calculated from the density of corresponding liquid.

molecular volumes of bonded chains (from column 7, Table 2) with that for the corresponding ( $n+2$ )-alkanes calculated from their density. Both dependencies show approximately parallel slopes. Experimental effective molecular volumes in the bonded phase are slightly lower than those for liquid alkanes. This indicates a slightly more compact molecular arrangement of bonded ligands on the silica surface compared to what is observed in the liquid state. The slope of both lines are parallel with the distance of  $\sim 35 \text{ \AA}^3$  between each line. This indicates that the volume of main alkyl chain is actually the same as in the liquid phase and the difference is due to the volume of the anchoring group.

Data from two publications [3,7] allow correct recalculation of the effective molecular volume of the attached ligands. These molecular volumes together with those calculated from liquid density are shown in Fig. 5. As can be seen, these data also show reasonable correlation of surface molecular arrangement with that of the liquid alkanes. In addition the calculated effective molecular volume for bonded alkyl chains from three different research groups shows a reasonable degree of consistency, in spite of the possible experimental errors involved in the determination of the adsorbent pore volume, surface area of silica, and carbon load. These data (Figs. 4 and 5) show that the effective molecular volume of alkyl chains bonded to the silica surface corresponds to that volume of neat liquid alkanes.

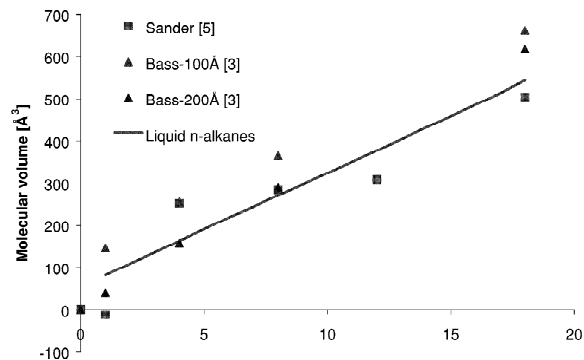


Fig. 5. Comparison of the molecular volumes of bonded ligands with the molecular volumes of liquid  $n$ -alkanes. ( $n$ -Alkane volumes are plotted in  $n+2$  scale to account for the two additional methyl groups of the bonded ligands).

Silica modified with trimethylchlorosilane ( $C_1$ ) has bonding density of  $4.16 \mu\text{mol}/\text{m}^2$ ,  $C_{18}$  bonded phase had a bonding density of  $2.51 \mu\text{mol}/\text{m}^2$ . These values are translated to the approximate linear distance between anchoring points of  $4.3 \text{ \AA}$  for  $C_1$  and  $7 \text{ \AA}$  for  $C_{18}$  on the surface. Fig. 6 shows the molecular model of silica surface with  $C_1$  (A) and  $C_{18}$  (B) chains attached on the surface with mentioned average distance. The  $C_1$  layer is dense and does not have any “gaps” between bonded chains.

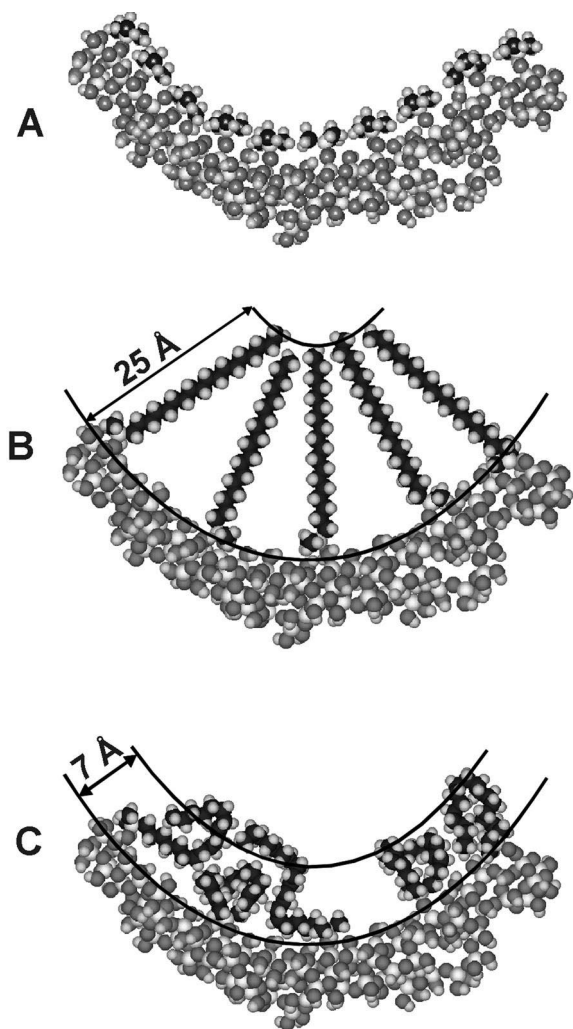


Fig. 6. Model of the silica surface modified with (A) trichlorosilane ( $C_1$ ), octadecylsilane (B) in all-*trans* conformation ( $C_{18}$ ), and (C) the same  $C_{18}$  with minimized energy.

The  $C_{18}$  bonded layer, on the other hand, has significant space between bonded chains shown in all-*trans* conformation, which in further discussion is denoted as “free” volume. Model C in Fig. 6 represents the same  $C_{18}$  bonded phase in a “collapsed” state. The energy of these chains has been minimized using MM2 minimization with HyperChem software. The density of this phase with minimized energy is the same as the density of the corresponding liquid *n*-alkanes.

The shortest ligand ( $C_1$ ) has a very high bonding density. Its effective volume is mainly determined by bonding density and not by its conformational freedom, which is minimal. However, longer ligands ( $C_8$ – $C_{18}$ ) have lower bonding density values (Table 1, column 6) and greater conformational freedom (Fig. 6B and C). Thus, their effective volumes are determined not only by their bonding density but also by their conformation. The correspondence of the effective molecular volume assessed from LTNA data with that volume of liquid *n*-alkanes (Fig. 4) indicates that these flexible chains most probably in the most dense conformation (Fig. 6C) and may fill “gaps” in the surface.

Since adsorbent samples were vacuumed first at elevated temperature and then submerged to liquid nitrogen temperature (77 K), which restricts the chain mobility, the experimental dependencies represent the molecular arrangement of alkyl chains in vacuum. However, the calculation has been made solely by using the surface area of bare silica and the total pore volume of modified adsorbents, without any assumptions in reference to the pore shape, structure and the pore size distribution.

This treatment leads to the conclusion that alkyl chains under vacuum are “collapsed” on the surface, so that they occupy minimum possible volume, and surface energy is minimized.

However, after being exposed to mobile phase under HPLC conditions, the conformation (or bulk molecular arrangement) that these ligands are in is another question. We will address this later in this paper.

### 3.2. Bonded layer thickness and bonding density

In the previous section it was shown that the bonded layer has dense molecular arrangement simi-

lar to that in corresponding liquid *n*-alkanes. The thickness of that layer could be obtained from the bonded layer volume per unit area (column 5, Table 2) by simple unit conversion. The experimental dependence of the bonded layer thickness on the number of carbon atoms in bonded chains is shown in Fig. 7. The theoretical maximum bonded layer thickness, estimated as a length of the corresponding alkyl chains in an all-*trans* conformation, is also plotted in Fig. 7.

As is seen from Fig. 7A, the theoretical and experimental thickness of the bonded layer is the same for trimethylsilane ( $C_1$ ) bonded on the surface. This is logical since there is minimal variation in the conformation of this ligand. All other ligands due to their conformational freedom may “collapse” to form the more compact layer and hence show a reduced bonded layer thickness compared to theoretical values. The difference between the theoretical maximum thickness of the bonded layer and the experimental thickness, multiplied by the surface area of the silica represents the “free” volume theoretically available for analyte molecules to penetrate, as seen in Fig. 7B. A corresponding model is shown in Fig. 6B.

For the adsorbents modified with the short-chain ligands,  $C_1$ – $C_4$ , analytes cannot have partitioning-based retention, since there is not enough free

volume between bonded chains. For  $C_{18}$  modified adsorbents, partitioning could be significant, provided that the bonded chains prefer to interact with eluent and analyte molecules, rather than with each other. If partitioning of analyte molecules within the long chains is a major retention mechanism there should be a significant difference in the retention mechanism between short- and long-chained reversed-phase adsorbents. Partitioning of analytes into the alkyl chains can only occur if these chains are highly solvated and extended away from the surface. If not, the predominant retention mechanism would be adsorption on top of the collapsed bonded layer and would be independent of the alkyl chain length. However, even if the chains were collapsed, the retention process would be dependent on the degree of shielding of underlying polar silanols, which would be greater for longer bonded alkyl chains.

An alkyl bonded layer may have different conformational configurations when exposed to different organic solvents. It would be logical to expect different degrees of solvation, if any, of bonded alkyl chains with THF, acetonitrile and methanol. If bonded chains are solvated with organic molecules their conformation would have more extended shape (Fig. 6B) and pore volume will be decreased compare to that volume measured using LTNA method. If there is no solvation, bonded chains are in

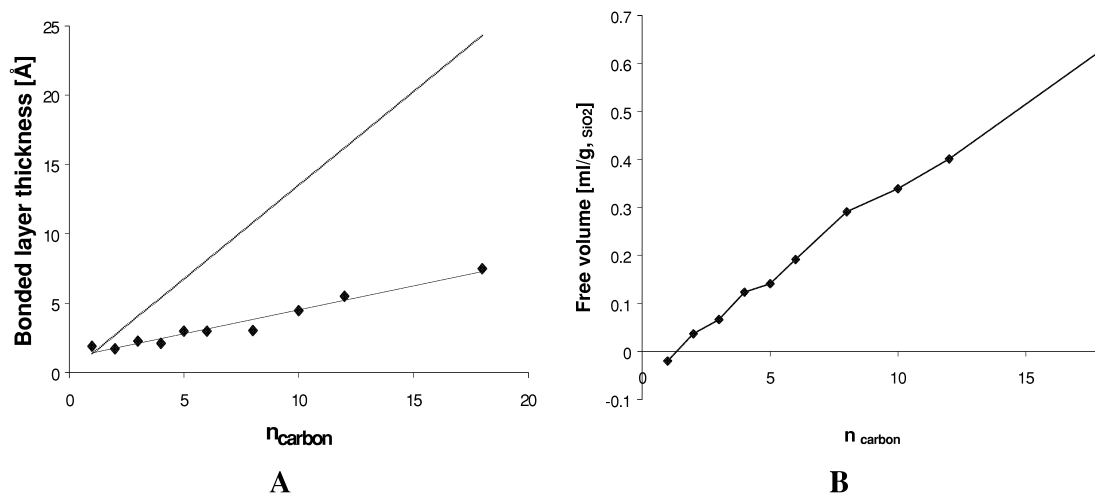


Fig. 7. (A) Comparison of experimental bonded layer thickness dependence (bottom line) on the number of carbon atoms with theoretical (top line) calculated for all-*trans* alkyl chains conformation. (B) Hypothetical “free volume” in the bonded layer calculated as a difference between theoretical and experimental thickness multiplied by specific surface of silica.



collapsed state and adsorbent pore volume will be the same as it is from LTNA experiments (Fig. 6C).

To evaluate these possibilities pore volume of modified adsorbents under HPLC conditions must be measured and compared with values measured under LTNA conditions. The volume of liquid phase in the column (void volume,  $V_0$ ) consists of the volume inside the adsorbent pores ( $V_p$ ) and the volume between adsorbent particles ( $V_{ex}$ ).  $V_{ex}$  can be measured as an exclusion volume by a GPC technique with high-molecular-mass polymers. The difference between the void volume and the exclusion volume is a measure of the pore volume in the column.

### 3.3. Determination of the void volume

The void volume of each column was obtained with three different methods: minor disturbance method [19,20], deuterated eluent components [17,21] and pycnometry [18,20]. In the minor disturbance method, the total volume of the liquid phase in the column ( $V_0$ ) is measured as an integral average of the minor disturbance peak retention dependence on the eluent concentration (Fig. 3) [19]. According to Riedo and Kováts [21] and Knox and Kaliszan [17] the retention volume of the deuterium-labeled compound of a single component eluent should correspond to the column void volume. Thus, the void volumes for each column was also obtained by determination of the retention of deuterated acetonitrile ( $C^2H_3CN$ ) eluted with pure acetonitrile. Void

volumes were also determined by pycnometry, “the weighing method” (McCormick and Karger [20] and Slaats et al. [18]). In the pycnometry method, void volume is determined by weighing a column, which is consecutively filled with two different pure eluents of different densities at a constant temperature. The column is first flushed with an eluent of a certain density,  $d_a$ , then weighed,  $w_a$ . Then a second eluent of different density,  $d_b$  is flushed through the column and the column is reweighed,  $w_b$ . The void volume is determined from Eq. (3) using the two column masses filled with the different liquids and the corresponding densities of liquids:

$$V_0 = \frac{w_a - w_b}{d_a - d_b} \quad (3)$$

The void volumes using the weighing method were determined with two different sets of eluents: (1) acetonitrile and methylene chloride and (2) THF and acetonitrile.

The minor disturbance measurements were performed using acetonitrile–water, methanol–water, and tetrahydrofuran–water eluents for all columns studied (experimental data are shown in Appendix Tables A.1–A.5). Comparison of the minor disturbance retention dependencies on concentration for all three eluent systems is shown in Fig. 3. Void volume values were calculated as an integral average of measured dependencies of the minor disturbance peaks, according to the procedure described in Ref. [19]. These values for the whole series of columns

Table 3

Void volume values measured with MeCN–water, MeOH–water and THF–water and labeled component for first set of columns (in ml)

Chain length	$V_{\text{labeled}}^b$ , from MeCN	$V_{\text{md}}^c$			RSD (%)
		MeCN–water	MeOH–water	THF–water	
1	1.914	1.893	1.954	2.015 <sup>a</sup>	2.76
2	1.895	1.909	1.951	1.974 <sup>a</sup>	1.90
3	1.850	1.838	1.86		0.6
4	1.885	1.876	1.898	1.910 <sup>a</sup>	0.79
5	1.860	1.846	1.880		0.92
6	1.833	1.818	1.847		0.79
8	1.827	1.814	1.869	1.865 <sup>a</sup>	1.49
10	1.811	1.797	1.825		0.77
12	1.770	1.765	1.807		1.29
18	1.723	1.713	1.751	1.718 <sup>a</sup>	0.98

<sup>a</sup> Data from the second set of columns.

<sup>b</sup> Retention volume of deuterated acetonitrile eluted using pure acetonitrile flow.

<sup>c</sup> Integral average of the minor disturbance peak retention dependence on the eluent concentration (Fig. 3).

Table 4

Void volume values measured with MeCN–water, MeOH–water and THF–water, pycnometry, labeled component for second set of columns

Chain length	Pycnometry (MeCN–MeCl <sub>2</sub> )	Pycnometry (MeCN–THF)	$V_{\text{labeled}^*}$ from MeCN	MeCN–water	MeOH–water	THF–water	RSD (%)
1	1.913	1.917	1.975	1.956	1.978	2.015	1.87
2	1.884	1.880	1.937	1.955	1.951	1.974	1.90
4	1.827	1.815	1.884	1.912	1.898	1.910	2.11
8	1.777	1.800	1.834	1.845	1.830	1.865	1.61
18	1.648	1.709	1.691	1.693	1.694	1.718	1.31

are shown in Table 3 as well as the values determined by the deuterium labeled component. Table 4 shows the void volume values calculated for the second set of columns from the minor disturbance method, pycnometry and deuterated labeled component.

As can be seen from Table 3, void volume values measured with different eluents correlate well for each column studied. Table 3 also shows that column void volumes consistently decrease with an increase of the bonded chain length. The dependence of the void volume on the number of carbon of the alkyl modifier in three different eluents is shown in Fig. 8. The difference between the void volumes of the C1 and C<sub>18</sub> columns is about 0.3 ml (Fig. 8) which correlates well with the value for the expected pore volume decrease after surface modification with alkyloctadecylsilane, as estimated in the Introduction.

Deviations in column packing density could also account for differences in the void volumes for columns studied. In order to determine if the de-

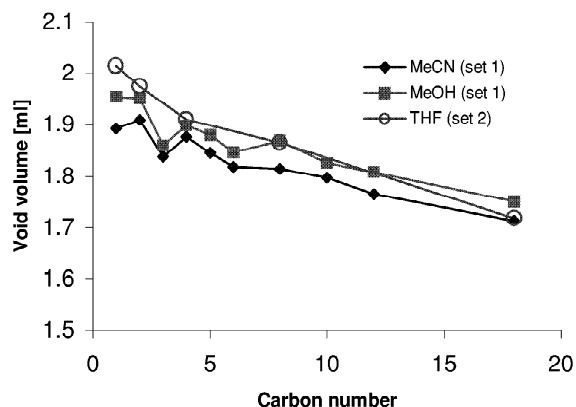


Fig. 8. Decrease of the column void volume with the increase of the alkyl modifier chain length bonded on the silica surface.

crease of the void volume is due to a decrease in the pore volume or differences in the packing density, the packing density must be determined.

### 3.4. Determination of column exclusion volume and packing density

Column exclusion volume ( $V_{\text{ex}}$ ), obtained from GPC experiments, is actually a measure of the interparticle volume in the column. The original porous silica used has an average pore diameter of 97 Å (Table 1). In order to obtain a correct value of the total exclusion volume, a series of GPC experiments with polystyrene (PS) standards of different molecular masses were run. The chosen PS standards had molecular masses of 97 200, 194 000, 470 000, 860 000, 1 840 000, and 2 700 000, to ensure their exclusion from the adsorbent pores. THF was used as the solvent. Normally, the higher the analyte molecular mass, the lower its retention volume, even in the total exclusion region (when molecules does not penetrate in the adsorbent pore space). This is associated with the fact that the retention volume

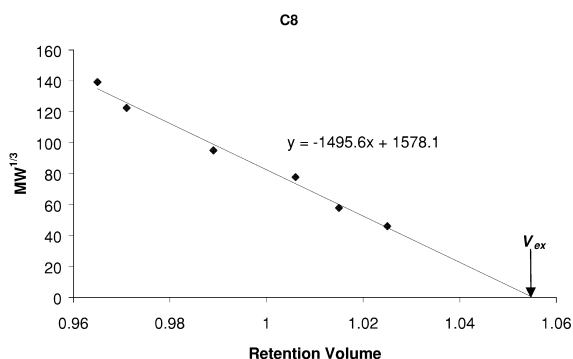


Fig. 9. Decrease of the retention volume due to the molecular size on C<sub>8</sub> modified silica.

Table 5  
Column exclusion volumes and packing density (first set)

Chain length	$V_{\text{ex.}}$ (ml)	$d_{\text{packing}}$ (%, v/v)
1	1.034	58.5
2	1.034	58.5
3	1.013	59.3
4	1.029	58.7
5	1.041	58.2
6	1.026	58.8
8	1.037	58.4
10	1.030	58.7
12	1.031	58.6
18	1.032	58.6
RSD (%)	0.729	0.703

corresponds to all possible positions of the middle mass points of the molecular globe (excluding the globe radius). The mean-square size of a linear polymer in solution of is proportional to the cubic root of its molecular mass [22]. The dependence of retention volume versus the cubic root of the molecular mass of the corresponding excluded polymer, should be linear. This was found to be the case for all columns studied, as shown in Fig. 9.

The column exclusion volume was found by extrapolation of the exclusion branch of the GPC curve to zero mass point, as is shown in Fig. 9 where:

$$V_{\text{ex.}} = -(\text{intercept/slope}) \quad (4)$$

Exclusion volume values for the 10 columns of set 1 are shown in Table 5. The packing density was calculated as:

$$d_{\text{packing}} (\%) = \frac{V_{\text{column}} - V_{\text{ex.}}}{V_{\text{column}}} \cdot 100 \quad (5)$$

where  $V_{\text{column}}$  is the volume of the empty column and  $V_{\text{ex.}}$  is the measured exclusion volume. The packing density of all columns (Table 5) is very consistent and has an average value of 58.6% of the column volume with a 0.7% RSD. This confirms that the decrease of the column void volume with the increase of the bonded chain length is solely due to the decrease of the adsorbent pore volume.

### 3.5. Correlation of the column void volume with the adsorbent pore volume

Modification of the silica surface does not significantly change the diameter of the adsorbent particles. For example, the length of a  $C_{18}$  chain in its all-*trans* conformation is about 25 Å [7]. A 25 Å increase on the outside surface of a 5 µm particle makes only a 0.05% difference in particle diameter. The difference of the volume of empty column ( $V_{\text{col.}}$ ) and the exclusion volume ( $V_{\text{ex.}}$ ) represents the total volume of all adsorbent particles in the column

Table 6  
Comparison of the adsorbents porosity measured by LTNA and HPLC methods

1: Adsorbent	2: $V_{\text{part.}}^{\text{tot.}}$ ; $V_{\text{col}} - V_{\text{ex.}}$ (ml/column)	3: $V_{\text{pore}}^{\text{tot.}}$ ; $V_0 - V_{\text{ex.}}^a$ (ml/column)	4: Adsorbent porosity (HPLC)	5: $V_p^{\text{Si}}$ (LTNA) (ml/g)	6: LTNA/HPLC pore volume ratio
$C_1$	1.458	0.860	0.590	0.894	1.040
$C_2$	1.458	0.875	0.600	0.901	1.030
$C_3$	1.479	0.825	0.558	0.880	1.067
$C_4$	1.463	0.847	0.579	0.889	1.049
$C_5$	1.451	0.805	0.555	0.854	1.061
$C_6$	1.465	0.792	0.540	0.853	1.077
$C_8$	1.454	0.776	0.534	0.853	1.098
$C_{10}$	1.462	0.767	0.525	0.811	1.056
$C_{12}$	1.460	0.734	0.502	0.760	1.036
$C_{18}$	1.459	0.681	0.466	0.685	1.007
				RSD (%)	2.5

<sup>a</sup>  $V_0$  values used were from minor disturbance method with MeCN–water.

Table 7  
Correlation of the calculated and measured mass of the adsorbents in the column

1: Adsorbent	2: $V_p$ , from LTNA (ml/g)	3: $V_{ex}$ , from GPC (ml)	4: Adsorbent mass (g)	5: $V_0^a$ , from HPLC (ml)	6: $V_0$ , calculated (ml)
$C_1$	0.804	1.035	1.117	1.956	1.933
$C_2$	0.804	1.041	1.142	1.955	1.959
$C_4$	0.778	1.030	1.125	1.912	1.905
$C_8$	0.727	1.026	1.150	1.845	1.862
$C_{18}$	0.531	1.005	1.269	1.693	1.679

<sup>a</sup>The  $V_0$  values used were determined with the minor disturbance method using MeCN–water.

( $V_{part}^{tot.}$ ), shown in Eq. (6). The difference of the column void volume ( $V_0$ ) and interparticle volume represent the total pore volume ( $V_p^{tot.}$ ) in these particles, shown in Eq. (7):

$$V_{col.} - V_{ex.} = V_{part}^{tot.} \quad (6)$$

$$V_0 - V_{ex.} = V_p^{tot.} \quad (7)$$

The ratio of the total pore volume per column (Table 6, column 3) to the total particle volume (Table 6, column 2) represents the adsorbent porosity, as assessed by HPLC and these values are shown in Table 6 (column 4). The total volume of adsorbent particles in the column is proportional to the mass of silica in the column. It was discussed

above that chemical surface modification alters only the pore volume and not the particle volume. This allows the comparison of the porosity assessed using HPLC with the porosity assessed using LTNA.

If the conformation of the bonded alkyl chains under HPLC conditions is the same as it is under LTNA conditions, then the pore volume from LTNA in reference to silica ( $V_p^{Si}$ ) is proportional to the total pore volume from HPLC, and the proportionality coefficient should be the same for all adsorbents. Indeed, for  $C_1$  the pore volume should be the same for HPLC and LTNA, due to the lack of conformational freedom of the alkyl group. However, for a  $C_{18}$  modified adsorbent, the situation may be different, since long alkyl chains may have different conformations under LTNA and HPLC conditions,

Table A.1. MeCN–water minor disturbance retention volumes for first set of columns

Concentration MeCN (% v/v)	$C_1$	$C_2$	$C_3$	$C_4$	$C_5$	$C_6$	$C_8$	$C_{10}$	$C_{12}$	$C_{18}$
0	2.694	2.927	2.659	2.817	3.059	3.282	3.496	3.671	4.020	4.258
1	2.373		2.480		2.776	2.836	2.906	3.019	3.089	3.125
5	2.309	2.481	2.438	2.557	2.582	2.607	2.589	2.586	2.530	2.432
10	2.328	2.464	2.406	2.467	2.466	2.447	2.438	2.451	2.386	2.274
20	2.270	2.316	2.259	2.297	2.267	2.230	2.236	2.255	2.210	2.122
30	2.131	2.125	2.074	2.097	2.053	2.011	2.001	1.983	1.964	1.903
35	2.022	2.001	1.966	1.967	1.918	1.872	1.865	1.833	1.800	1.751
40	1.918	1.852	1.837	1.807	1.805	1.746	1.742	1.696	1.683	1.628
50	1.636	1.606	1.552	1.567	1.533	1.486	1.491	1.448	1.410	1.381
60	1.488	1.445	1.397	1.397	1.390	1.354	1.352	1.318	1.268	1.256
70	1.473	1.431	1.387	1.387	1.373	1.350	1.342	1.322	1.283	1.244
80	1.589	1.543	1.491	1.497	1.473	1.442	1.439	1.409	1.376	1.324
90	1.789	1.721	1.667	1.677	1.648	1.607	1.609	1.580	1.543	1.476
95	1.927	1.839	1.790	1.797	1.776	1.747	1.736	1.715	1.673	1.597
99	2.227		2.090		2.075	2.088	2.063	2.005	1.985	1.921
100	3.027	2.932	2.838	2.867	3.097	3.415	2.770	2.934	3.224	3.540

and organic eluents may show preferential solvation of the bonded layer. The ratio of the total pore volume assessed by HPLC (Table 6, column 3) and the pore volume determined by LTNA per gram of  $\text{SiO}_2$ ,  $V_p^{\text{Si}}$  (Table 6, column 5) is shown in column 6 of Table 6. The ratio of HPLC to LTNA pore volume is very consistent for all columns, with a 2.5% RSD. This clearly indicates that prevalent alkyl chain conformation of the bonded phase exposed to HPLC eluent is practically the same (in terms of occupied volume) as it is under LTNA conditions. Therefore, alkyl chains tend to occupy lowest possible volume (“collapsed”) since the intermolecular interactions are dominant compared to eluent–alkyl chain interactions.

This conclusion is also supported by the comparison of the void volume values obtained for three different eluent systems. If there is preferential solvation of the bonded phase the pore volume decreases and hence yield lower pore volume values. As can be seen from Table 3,  $V_0$  values measured with methanol, acetonitrile, and tetrahydrofuran match for each column and are independent of the eluent used.

### 3.6. Relation of the column void and exclusion volume to the adsorbent pore volume

The relationship between the void volume, exclusion volume, and the adsorbent pore volume is also expressed in the form of Eq. (8), if the mass of the adsorbent in the column is known. The column void volume is equal to the sum of the exclusion volume and the product of the adsorbent specific pore volume and the mass of the packing material in the column:

$$V_p m_a + V_{\text{ex.}} = V_0 \quad (8)$$

where  $V_p$  is the specific pore volume of used adsorbent,  $V_{\text{ex.}}$  is column exclusion volume obtained from GPC experiments,  $V_0$  is a column void volume, and  $m_a$  is a mass of adsorbent packed in the column. Very good correlation of the void volume values measured with three different eluents suggests that the conformation of the alkyl chains on the surface does not change significantly and most probably maintains the same conformation as it was under vacuum conditions (LTNA).

In order to further test this hypothesis several of the studied columns (set 2) were unpacked, the packing material collected, dried to constant mass under vacuum and weighed. Thus all four values in Eq. (8) are obtained by independent methods. The adsorbent pore volume was measured by LTNA (vacuum conditions), the column exclusion volume was measured using GPC, the column void volume was measured using deuterated components and the minor disturbance method (HPLC conditions), and the adsorbent mass was directly weighed; all data are shown in Table 7 columns 2–5.

Expected void volume values were calculated from Eq. (8) for each column using adsorbent pore volume from LTNA, measured adsorbent mass, and interparticle volume from GPC. Comparison of the void volume values obtained from Eq. (8) using LTNA data (Table 7, column 6) and HPLC measured column void volumes (Table 7, column 5) shows very good correlation, in fact the deviation does not exceed 2.5%.

This confirms that alkyl chains bonded on the surface of porous silica occurs in their most dense “collapsed” conformation under HPLC conditions, under any of the solvents studied. Since adsorbent pore volume is the same under LTNA and HPLC conditions, this also suggests that no significant solvation occur with any of the solvents studied.

## 4. Conclusions

In this paper, the geometry of reversed-phase HPLC adsorbents is described. It is shown that specific pore volume of alkyl modified HPLC adsorbents measured with LTNA method are consistent with pore volumes determined under HPLC conditions. The void volume, one of the most important characteristics of HPLC columns has been measured by three different methods (minor disturbance, retention of isotopically labeled components, and pycnometry). All three methods show similar results with RSD less than 2%. These methods allow the measurement of the total volume of the liquid phase in the column without distinction if the liquid is moving, stagnant or retained at close proximity to the surface due to viscosity or surface interactions. This approach is the most universal one, since it

Table A.2. Acetonitrile–water minor disturbance retention volumes for second set of columns

Concentration MeCN (% v/v)	C <sub>1</sub>	C <sub>2</sub>	C <sub>4</sub>	C <sub>8</sub>	C <sub>18</sub>
0	2.957	2.995	3.014	3.715	4.316
1	2.440	2.530	2.669	3.044	3.041
5	2.388	2.523	2.568	2.640	2.379
10	2.388	2.506	2.483	2.478	2.204
20	2.324	2.371	2.309	2.229	2.069
30	2.176	2.177	2.105	1.999	1.871
35	2.078	2.061	1.975	1.855	1.723
40	1.949	1.920	1.836	1.717	1.583
50	1.683	1.637	1.567	1.473	1.361
60	1.528	1.484	1.415	1.333	1.253
70	1.525	1.482	1.406	1.325	1.244
80	1.655	1.595	1.531	1.434	1.325
90	1.858	1.779	1.709	1.601	1.478
95	2.023	1.933	1.877	1.776	1.624
99	2.508				2.062
100	3.170	3.126	3.580	3.434	3.360

Table A.4. Methanol–water minor disturbance retention volumes for second set of columns

Concentration MeOH (% v/v)	C <sub>1</sub>	C <sub>2</sub>	C <sub>4</sub>	C <sub>8</sub>	C <sub>18</sub>
0	2.247	2.244	2.250	2.436	2.435
1	2.201	2.222	2.245	2.376	2.364
5	2.105	2.125	2.186	2.173	2.012
10	2.065	2.090	2.073	2.035	1.879
20	2.02	2.034	1.985	1.901	1.744
30	1.998	1.974	1.911	1.819	1.676
40	1.935	1.912	1.844	1.755	1.629
50	1.918	1.881	1.804	1.740	1.601
60	1.906	1.848	1.788	1.706	1.582
70	1.906	1.844	1.785	1.705	1.576
80	1.916	1.860	1.798	1.715	1.584
90	1.943	1.886	1.834	1.748	1.607
95	1.972	1.931	1.873	1.783	1.635
99	2.042	2.049	1.987	1.893	1.785
100	3.389	3.531	2.794	3.010	2.792

allow a comparison of different systems, and it is similar to the convention “Nothing is adsorbed” introduced by Riedo and Kováts [21] for the description of adsorption systems.

Our results suggest, in spite of the existence of conformational freedom and definite presence of chain mobility (shown in previous investigations [23–26]) of bonded ligands, the average molecular arrangement in bonded layers remain very dense or “liquid like”. A similar indication on the dense conformation of bonded layer was obtained by

Sander et al. [27] from small angle neutron scattering. The penetration of significant amounts of organic eluent between these chains is unlikely. This conclusion is drawn from the similarity of the geometric parameters assessed using LTNA (vacuum conditions) and HPLC. This similarity was observed for all chemically modified adsorbents, from C<sub>1</sub>–C<sub>18</sub>, that were studied.

We show that for the determination of void volume the most practical and convenient method is the injection of isotopically labeled sample of a

Table A.3. Methanol–water minor disturbance retention volumes for first set of columns

Concentration MeOH (% v/v)	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>	C <sub>6</sub>	C <sub>8</sub>	C <sub>10</sub>	C <sub>12</sub>	C <sub>18</sub>
0	2.229	2.187	2.152	2.222	2.280	2.339	2.434	2.431	2.492	2.521
1	2.149	2.149	2.110	2.178	2.235	2.285	2.339	2.342	2.359	2.324
5	2.093	2.069	2.051	2.093	2.132	2.140	2.174	2.139	2.151	2.103
10	2.059	2.045	2.002	2.049	2.055	2.042	2.078	2.020	2.013	1.947
20	2.021	1.992	1.954	1.985	1.949	1.914	1.945	1.896	1.877	1.812
30	1.977	1.901	1.871	1.904	1.876	1.825	1.874	1.803	1.802	1.736
40	1.929	1.870	1.830	1.862	1.807	1.780	1.819	1.759	1.747	1.688
50	1.892	1.840	1.810	1.836	1.794	1.764	1.779	1.740	1.718	1.664
60	1.875	1.809	1.774	1.829	1.768	1.734	1.753	1.720	1.696	1.643
70	1.867	1.802	1.766	1.797	1.764	1.732	1.747	1.697	1.693	1.642
80	1.889	1.826	1.784	1.808	1.780	1.736	1.771	1.711	1.706	1.649
90	1.917	1.853	1.796	1.829	1.801	1.758	1.793	1.727	1.730	1.677
95	1.935	1.881	1.820	1.876	1.811	1.792	1.816	1.769	1.751	1.701
99	2.060	1.963	1.857	1.893	2.292	2.118	1.946	2.022	1.802	1.764
99.5					2.382	2.379		2.261		
100	2.654	3.105	2.363	3.173	3.543	3.226	2.202	3.321	2.176	2.083

Table A.5. THF–water minor disturbance retention volumes for second set of columns

Concentration THF (% v/v)	C <sub>1</sub>	C <sub>2</sub>	C <sub>4</sub>	C <sub>8</sub>	C <sub>18</sub>
0	5.428	6.138	6.863	8.63	5.884
1	2.922	3.568	4.334	5.161	4.485
5	2.710	3.127	3.039	2.856	2.605
10	2.561	2.539	2.416	2.344	2.264
20	2.384	2.197	2.139	2.084	2.030
30	2.151	2.050	1.960	1.893	1.759
40	1.950	1.89	1.782	1.696	1.514
50	1.731	1.685	1.584	1.501	1.320
60	1.569	1.532	1.427	1.374	1.215
70	1.497	1.472	1.387	1.256	1.161
80	1.562	1.499	1.430	1.330	1.187
90	1.750	1.648	1.568	1.480	1.307
95	1.939	1.814	1.717	1.639	1.391
99	2.532	2.239	2.233	2.004	1.774
99.5				7.650	2.824
100	6.874	5.871	5.534	7.596	5.884

single component eluent. Injection of deuterated acetonitrile with regular acetonitrile as the eluent is probably the most practical. One of the advantages is that the measured void volume value is independent of the amount injected.

We also report a convenient method for estimating the actual amount of the adsorbent packed into the column, without actually unpacking it. This is done by measuring the exclusion volume (GPC); column void volume (labeled component) and obtaining the adsorbent pore volume (value could be obtained from the column manufacturer).

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## Appendix A

Experimental results on the retention of minor disturbance peaks of different eluents on all columns (Tables A.1–A.5)

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