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Interpretation of the excess adsorption isotherms of organic eluent components on the surface of reversed-phase phenyl modified adsorbents

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Abstract

The adsorption of three organic eluent components (acetonitrile, methanol, and tetrahydrofuran) from water were measured on four phenyl-type bonded phases using the minor disturbance method. The thicknesses of organic layer enriched above the phenyl-type bonded ligands were assessed and interpreted. Acetonitrile and tetrahydrofuran showed multilayer formation while methanol showed monomolecular adsorption. These results were compared to those obtained on alkyl bonded phases.

Keywords: Adsorption; Reversed-phase; Phenyl-type bonded phases

1. Introduction

Phenyl-modified HPLC stationary phases have attracted an increasing attention in the last decade. The phenyl-bonded phases have been used successfully to resolve positional isomers [1,2], tocopherols [3], flavonoids [4,5] (plant extracts), taxols [6–9] and their closely related impurities. Phenyl type phases with their hydrophobic π–π active aromatic moieties may introduce an additional component to the retention of aromatic analytes. Therefore solutes with π systems will display a different retention behavior on π containing stationary phases compared to alkyl bonded phases.

Nakashima et al. [10] attempted to confirm the existence of π–π interactions in reversed phase HPLC conditions through separation of polycyclic aromatic hydrocarbons (PAH) on silicas modified with amino-propyl-silyl ligands and several amino-propyl-silyl ligands derivatized with heterocyclic moieties. The retention of the PAHs increased as π-electron densities of the amino-propyl-silyl derivatized bonded ligands increase, while analytes possessing no π-electrons in the bonded ligands were barely retained on the amino-propyl-silyl phase. These effects in PAH retention were attributed to π–π interactions between the stationary phases and the analytes under the conditions studied. Reubsaet and coworkers [11] estimated the strength and contribution of aromatic-aromatic interactions to the overall retention process. The retention behavior of uncharged aromatic and non-aromatic (steroids) analytes on alkyl bonded adsorbents and polystyrene cross-linked with divinylbenzene adsorbents (PS-DVB) were studied. They observed the retention of both sets of analytes increase with increasing hydrophobicity (log P) when eluted on silica based alkyl-bonded adsorbent under reversed phase conditions. Despite having the same log P_S, higher concentration of acetonitrile was required to elute aromatic compounds such as toluene than non-aromatic species (testosterone) on the PS-DVB bonded phases. The authors concluded, aside from hydrophobic interactions, π–π interactions also play a significant role in added capabilities of retaining aromatic analyte on aromatic bonded stationary phases. Horak and coworkers [12] found that for large

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polyaromatic compounds with conjugated aromatic systems, the π–π interaction becomes more dominant, while for small aromatic compounds hydrophobic properties are predominant.

Phenyl type stationary phases also could show differences in selectivity and retention when switching between methanol and acetonitrile modifiers. Acetonitrile is an electron rich organic modifier, which could suppress the π–π interactions between the solute and the aromatic moiety of the stationary phase. Methanol on the other hand does not contain π-electrons and analyte retention would primarily be based on hydrophobic interactions [13–15].

It is generally recognized that the type of organic eluent modifier plays dominant role in separation selectivity [16] although the mechanism of its influence on the analyte retention is a subject of intense investigation. The important part of this mechanism is the adsorption of the eluent components on the adsorbent surface. Eluent components adsorption behavior has been studied for many years [17–19] and many experimental adsorption isotherms have been reported [20–22] for most solvents used in HPLC. The application of these isotherms in regards to the interpretation of the retention of different analytes, on the other hand, is minimal and no accepted concept on the correlation of organic modifier adsorption isotherm with chromatographic retention and selectivity is available. This is probably due to the difficulties in interpreting adsorption data expressed in surface specific units. Indeed, commonly used retention factor, k, is a ratio of the adjusted retention volume, $V_R - V_0$, to the column void volume, V_0

$$k = \frac{VR - V0}{V0} \tag{1}$$

Assuming a partitioning retention mechanism the solution of the mass balance equation [23] leads to the following basic retention equation

$$V_R = V_M + V_S K \tag{2}$$

where V_M is the volume of the mobile phase in the column, V_S is a volume of the stationary phase in the column, and K is an equilibrium constant, which is an exponent of the Gibbs free energy of the analyte partitioning between these two phases (V_m and V_s). It would be convenient to get a simple relationship of chromatographically measured retention factor and thermodynamic energetic parameter of the system, but simple substitution of Eq. (2) into Eq. (1) leads to

$$k = \frac{V_M}{V_0} - 1 + \frac{V_S}{V_0} K \tag{3}$$

which is not a convenient expression since it contains three different volume parameters (mobile phase volume (V_M), stationary phase volume (V_S), and void volume (V_0). Only the assumption that $V_M = V_0$ leads to the commonly used relationship

$$k = \frac{V_S}{V_0} K \Rightarrow \ln(k) = \ln(K) + \ln\, \varphi \tag{4}$$

where $\phi = V_S/V_0$ is a phase ratio.

This assumption ($V_M = V_0$) is a critical one. It essentially defines a boundary between the mobile and stationary phases in the HPLC column and it also defines that the column void volume is only the volume of the mobile (moving) phase. The next question that arises is what is the stationary phase and where does the analyte actually get retained?

It is quietly accepted that in reversed-phase HPLC the bonded phase volume is the volume of the stationary phase [24,25]. This definition at first glance seems to work for bonded phases like C18-type phases with long chains attached on the surface, but on the other hand, for C1-type phases there is no volume available for the analyte to partition from the mobile phase (silica is a solid impermeable material and attached trimethylsiliyl groups are small and have no conformational freedom). There are also indications that even long-chain bonded phases are impermeable not only for the analyte, but also for the eluent molecules [26,27].

It is convenient to introduce the total volume of the liquid phase in the column (V_L) as a maximum volume where analyte molecules could actually reside. For any modified adsorbents this will be the sum of the interparticle volume and the pore volume, assuming the densest arrangement of bonded chains. This is essentially a definition of a column geometry parameter, which could be independently measured, using a gravimetric method, for example by weighing the dry column and the column filled with pure solvent of a known density, or by alternate methods such as minor disturbance method or injection of deuterated components [28]. Since the volume of liquid phase in the column is defined as maximum volume accessible for analyte molecules in the column then it includes the mobile phase and stationary phase volumes, or

$$V_L = V_M + V_S. \tag{5}$$

The correlation of analyte–stationary phase interactions with the chromatographic retention is commonly done via Eq. (4). This equation is derived for partitioning model of HPLC retention and requires the definition and determination of the stationary phase volume, which should be the same for all types of analytes irrespective to the analyte nature.

An alternative approach is based on the surface specific retention parameters and first was introduced by Kiselev [29]. Foti et al. [19] has strengthened the necessity of its application in HPLC. Analyte retention volume in this approach is expressed as

$$V_R = V_0 + SK_H \tag{6}$$

where S is the total adsorbent surface area in the column and K_H is essentially the analyte adsorption constant or more specifically the slope of the analyte excess adsorption

isotherm at infinitely small concentration (Henry constant), and V_0 here is the total volume of the liquid phase in the column. All parameters in Eq. (6) (V_0 and S) are independently measurable [26] and surface specific retention factor defined below is directly related to the Henry constant

$$k_s \equiv \frac{V_R - V_0}{S} = K_H \quad (7)$$

Surface specific retention factor is not dimensionless; it is expressed in ($\mu L/m^2$) and it can be positive or negative. If the analyte interactions with the adsorbent surface are weaker than the eluent interactions the analyte molecules will not be able to come close to the adsorbent surface (mainly occupied by adsorbed eluent molecules) and its retention volume will be smaller then V_0. This indicates that K_H is not a real thermodynamic equilibrium constant but rather the simple limit of the slope of the excess adsorption isotherm at infinite dilution.

Basic retention equation for a binary system is expressed as

$$V_R = V_0 + S\frac{d\Gamma}{dc} \quad (8)$$

where Γ is the excess adsorption isotherm of the analyte at concentration c. Detailed derivation of this expression is given in [30].

De Vault [23] followed by Kovats [31] discussed general differential mass balance in the column for a multicomponent system and concluded that an analytical solution is only available for a binary system. Most common chromatographic systems are essentially comprised of three-components where two components of binary eluent are present in significant concentrations and the analyte is several orders of magnitude lower in concentration (10^4 to 10^5 difference is the most common). This allows the assumption that the injection of the infinitively small quantity of the analyte does not disturb the adsorption equilibrium of the eluent components and thus it is possible to first describe their adsorption equilibrium and then use it for the independent description of the analyte retention. Corresponding expression for the analyte retention from binary eluent mixture was derived in [27].

Suggested approach requires the knowledge of the adsorption behavior of the binary eluent components of the selected adsorbent. In our previous paper [26] we report the adsorption isotherms of three main organic eluent modifiers (methanol, acetonitrile, and tetrahydrofuran) on the set of alkyl-modified silicas (from C1 to C18) and also verified the applicability of suggested model for the description of the HPLC retention of selected analytes. In this work we study the eluent adsorption behavior on the surfaces of phenyl-type bonded phases.

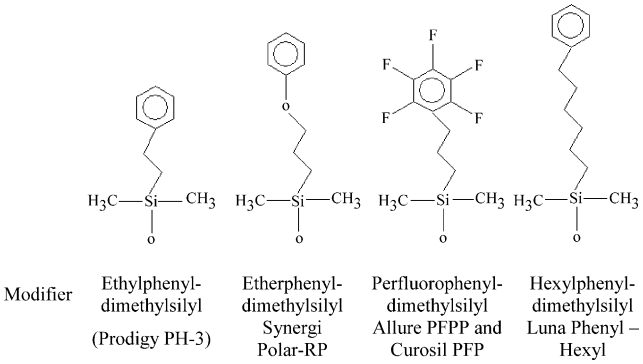

Fig. 1. The structures of the phenyl-bonded ligands.

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.

Detailed analysis of geometric characteristics of the columns used together with the discussion of the measurement procedure and practical applicability is given in our previous publication [26]. Table 2 lists all important parameters for the columns used.

2.2. 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,

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)	V_{pore} Silica (mL/g)	V_{pore} mod. (mL/g)	V_{pore} mod. (mL/g_{SiO_2})
Prodigy PH-3	9.9	344	5	9.68	163	2.69	0.97	0.687	0.791
Synergi Polar-RP	9.2	381	4	14.42	193	3.63	1.00	0.59	0.750
Curosil PFP	11.6	263	5	10.30	267	3.75	0.92	0.59	0.745
Allure PFPP	6.4	459	5	16.3	267	4.01	1.10	0.52	0.768
Luna Phenyl-Hexyl	11.0	357	5	17.54	219	3.79	1.00	0.51	0.658

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
Column parameters

Column	Prodigy PH-3	Synergi Polar RP	Curosil PFP	Allure PFPP	Luna Phenyl-Hexyl
Particle size (μm)	5	4	5	5	5
Void volume (mL)	1.881	1.716	1.767	1.650	1.694
S_{tot} (m^2)	378	385	292	413	400
$m_{ads.}$ (mod.) (g)	1.27	1.29	1.40	1.35	1.45
$m_{ads.}$ (silica) (g)	1.10	1.01	1.11	0.90	1.12

S_{tot} is a total surface area of base silica in the column.

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 in this section. 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.

Extra-column volumes for all HPLC systems used were measured by injection of 0.1 μL solution of benzene (10 ppm) without a column (connecting lines were directly connected using zero dead volume, ZDV, fitting). Average value of the first moment of the analyte peak retention volume measured in triplicate at 0.5; 1.0 and 1.5 mL/min eluent flow rate was used for the correction of all experimental measurements performed on corresponding HPLC system.

The experimental retention volumes of minor disturbance peaks used in the calculation of the void volumes and excess adsorption isotherms for the dynamic binary systems studied were shown in the Appendix A of the previous article [26]. In this study three binary systems of acetonitrile–water, methanol–water, and tetrahydrofuran–water were studied on four phenyl-type bonded phases. BET surface areas and pore volumes of the unmodified and modified silicas were determined using low temperature nitrogen adsorption with BET treatment [26]. Mean pore diameters and average particle sizes of the native unmodified silicas were obtained from Phenomenex and Restek. The geometric parameters of unmodified silicas are summarized in Table 1. Bonding densities of the phenyl-type ligands modified on corresponding silicas were determined from carbon elemental analysis (performed by Schwarzkopf Microanalytical Lab (Woodside, NY) using a Perkin Elmer 2400 CHN Analyzer using the ASTM method).

3. Result and discussion

Experimental values of the minor disturbance peak retention dependencies for acetonitrile–water, methanol–water, and tetrahydrofuran–water systems on all columns studied were reported previously [26] and used for the calculation of the column void volumes. Here we use the same experimental data for the calculation of the excess adsorption isotherms and interpretation of the adsorption behavior of common organic eluent components. Excess adsorption isotherms were calculated using the following equation [27]

$$\Gamma(c) = \frac{1}{S}\int_{o}^{c} (V_R(c) - V_0)\,dc \quad (9)$$

where $V_R(c)$ is the minor disturbance peak retention dependence on the composition of binary eluent; V_0 is the void volume, and S is the total surface area of the adsorbent in the column. Calculated adsorption isotherms for acetonitrile, methanol, and THF from water on studied adsorbents are shown in Figs. 2–4, respectively. Excess amount adsorbed represents the adsorbate quantity accumulated on the surface in excess to the quantity which would be on the same surface in the first instant the equilibrium solution is brought to contact with that surface (no adsorption has occurred yet).

The experimental determination of the excess adsorption does not require the definition or determination of the adsorbed layer volume (detailed discussion is given in [27]). However, this volume is needed for the interpretation of the adsorption isotherm [27,30,32].

The profile of the excess adsorption isotherm as a function of analyte equilibrium concentration (Figs. 2–4) shows an increase of the adsorbate accumulation on the surface up to approximately 40% (v/v) of the adsorbate in the equilibrium solution. At around 40% (v/v) the maximum of the excess

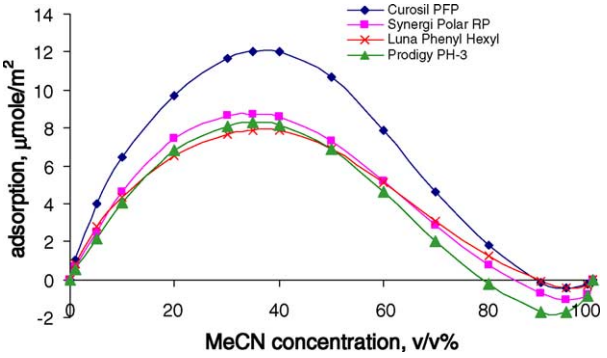

Fig. 2. Excess adsorption isotherms of acetonitrile from water on Prodigy PH-3, Synergi Polar RP, Curosil PFP, and Luna Phenyl-Hexyl bonded phases.

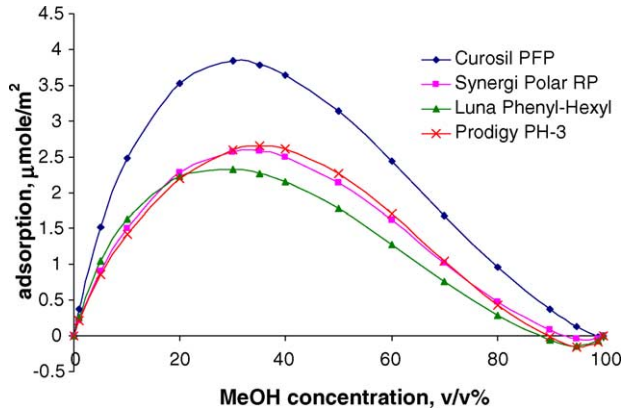

Fig. 3. Excess adsorption isotherms of methanol from water on Prodigy PH-3, Synergi Polar RP, Curosil PFP, and Luna Phenyl-Hexyl bonded phases.

amount adsorbed is observed. Further increase of the equilibrium concentration led to the steady decrease of the excessive adsorbed quantity until it reaches a zero value at 100% (v/v) of the adsorbate in the bulk liquid.

In the region between approximately 50 and 90% (v/v) of the adsorbate in bulk solution there is a linear decrease of the excess adsorption with increase of the bulk concentration. This essentially corresponds to the saturation of adsorbent surface with adsorbate and any increase of the equilibrium concentration will logically lead to the decrease of the excess, since there is no more room on the surface for additional adsorbate accumulation. Surface specific adsorbent capacity is the maximum quantity of the adsorbate which could be accumulated on the unit of the surface. Adsorbed layer volume could be estimated as a product of that maximum quantity of adsorbate on the surface and its molar volume. The state of complete filling of adsorbed layer is illustrated in Fig. 5, where the interface between the bulk solution and the adsorbed layer is essentially the hypothetical Gibbs dividing plane. The amount of the adsorbate in that layer could be represented as a sum of the excess adsorption value ($\Gamma(c_e)$) and the product of the equilibrium adsorbate concentration on the volume of adsorbed layer ($V_a \cdot c_e$). Using the surface

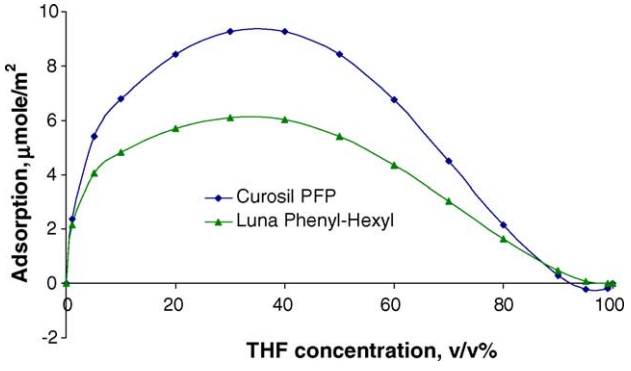

Fig. 4. Excess adsorption isotherms of tetrahydrofuran from water on Curosil PFP and Luna Phenyl-Hexyl bonded phases.

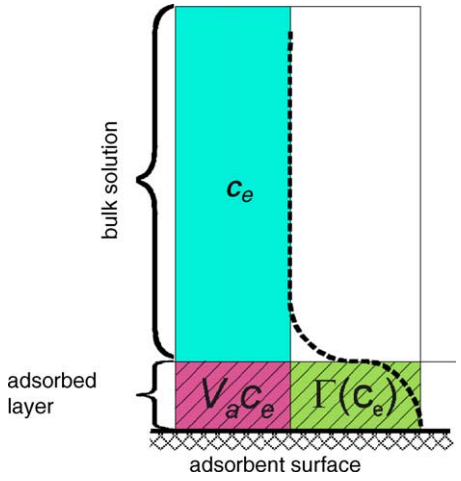

Fig. 5. Schematic representation of the static adsorption system in the state of a complete filling of adsorbed layer. Adsorbate accumulated on the surface is shown in striped region, the right part of this region is an excess amount adsorbed, and the left part is the amount which would be on the surface from the bulk solution even in the absence of the surface forces. Following Gibbs, the flat dividing plane is introduced, which divides bulk solution from the region where adsorbate is influenced by the surface forces of the adsorbent, while real adsorbate distribution is actually unknown and it may be shown as dashed line.

specific values (per 1 m^2) we can write

$$a_{\max.} = \Gamma(c_e) + c_e \cdot V_a \tag{10}$$

where $a_{\max.}$ is the maximum adsorbate amount which could be adsorbed on 1 m^2 on the adsorbent surface, $\Gamma(c_e)$ is the excess adsorption (in mole/m^2) at a given equilibrium concentration (c_e), and V_a is the surface specific volume of adsorbed phase.

Essentially Eq. (10) contains two unknown values: adsorption capacity ($a_{\max.}$) and adsorbed phase volume (V_a) or the surface specific volume of the adsorbed phase.

Expression 10 is only valid in the region of linear decrease of the excess adsorption isotherm when maximum adsorption capacity is actually achieved or, in other words, the whole adsorbed phase is filled with only adsorbate molecules. In this region the derivative of expression 10 will be

$$\frac{d\Gamma(c_e)}{dc_e} = -V_a \tag{11}$$

Therefore, the derivative of the excess adsorption isotherm in the region of a complete saturation of the adsorbed layer (maximum negative slope of the isotherm) is equal to the surface specific adsorbed layer volume. Surface specific adsorbed layer volumes (calculated relative to the surface of base silica) for all adsorbates on all adsorbents studied are shown in Table 3 along with the total adsorbed layer volumes. Comparison of the specific (per 1 g of base silica) adsorbed layer volumes from Table 3 with the specific adsorbent pore volume (per 1 g of bare silica, last column) from Table 1 show that these values are close for acetonitrile and THF

Table 3
Surface specific adsorbed layer volumes

Column	Luna-Phenyl-Hexyl		Curosil-PFP		Prodigy-3 Phenyl		Synergi-Polar-RP		Allure-PFPP	
	$\mu L/m^2$	mL/g[a]	$\mu L/m^2$	mL/g[a]	$\mu L/m^2$	mL/g[a]	$\mu L/m^2$	mL/g[a]	$\mu L/m^2$	mL/g[a]
MeCN	0.96	0.377	1.69	0.445	1.26	0.432	1.32	0.503	1.39	0.64
MeOH	0.224	0.08	0.355	0.093	0.286	0.098	0.251	0.096		
THF	1.13	0.403	1.845	0.485						

[a] mL/g_{SiO_2}.

adsorbates. In case of Allure-PFPP the adsorbed layer volume value is very close to the available pore volume, which means that on this adsorbent acetonitrile occupies the whole available porous space.

Estimation of the maximum adsorbed layer volume (from Eq. (11)) allows the calculation of the total adsorption isotherm. In general, for any equilibrium concentration the total adsorbed amount in the adsorbed layer defined in Eq. (11) is equal to the sum of the excess adsorption measured for the given equilibrium concentration ($\Gamma(c_e)$) and corresponding amount from the equilibrium solution ($V_a \cdot c_e$) and it could be written as a function of equilibrium adsorbate concentration

$$a_{tot.}(c_e) = \Gamma(c_e) + V_a \cdot c_e \quad (12)$$

Expression 12 is essentially equivalent to Eq. (10), except that Eq. (10) is only valid for the maximum negative slope of the excess adsorption isotherm and as such it was used for the determination of the maximum adsorbed layer volume. The assumption that this volume is the volume of adsorbed phase sets a model which we will use for the description of our adsorption system. This assumption divides the volume of the liquid phase in the column into the adsorbed layer volume and the volume of bulk liquid. Full (surface specific) adsorption isotherms on Luna Phenyl-Hexyl for all adsorbates studied are shown in Fig. 6.

On the left pane of Fig. 6(a) isotherms are shown in number of moles per meter square, while on the right pane (b) same isotherms are recalculated in terms of volume of the adsorbate on the surface ($\mu L/m^2$), assuming that adsorbate molar volume is constant. All isotherms show that at approximately 40% (v/v) of the adsorbate in the bulk solution the formation of adsorbed layer is practically complete and there are no significant changes in the adsorbed layer volume until greater than 95% (v/v). Between 95 and 100% (v/v) of the adsorbate in the bulk solution the slight increase of the total adsorption isotherm is observed. We associate this with the displacement of water adsorbed on strong adsorption sites, which are most likely accessible residual silanoles.

There is a noticeable difference in the adsorption behavior of methanol compared to acetonitrile and THF, such that its adsorption is approximately five times lower than acetonitrile and THF. This is essentially consistent with the same behavior observed previously for the adsorption of the same compounds on alkyl-modified adsorbents [27]. The adsorption of methanol is predominantly monomolecular, while acetonitrile and THF are adsorbed in a multilayered fashion.

The comparison of the maximum adsorbed layer volume of acetonitrile and methanol within the pore volume available in the column is given in Table 4. Acetonitrile occupies over 60% of the space available inside the adsorbent pores, while methanol fills up only 12% of that volume.

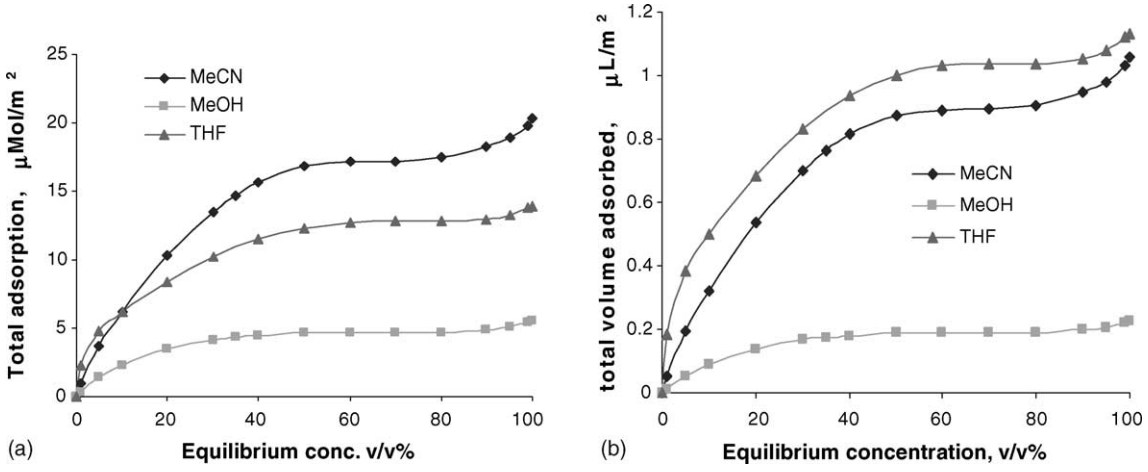

Fig. 6. Full adsorption isotherms of acetonitrile, methanol and tetrahydrofuran from water on Luna Phenyl-Hexyl adsorbent. Left pane is the total adsorption expressed in µmole/m^2, and right pane is the total adsorption in volume units ($\mu L/m^2$).

Table 4
Comparison of the column pore volume with the volume of the acetonitrile and methanol adsorbed layer, and percent of the pore filing with acetonitrile and methanol

	V_0 (mL)	V_{pore} (mL/column)	Acetonitrile (mL/column[a])		Methanol (mL/column[a])	
			$V_{ads.}$ layer	Percentage of V_{pore}	$V_{ads.}$ layer	Percentage of V_{pore}
Prodigy PH-3	1.846	0.88	0.48	55	0.11	12.5
Synergi Polar-RP	1.730	0.78	0.51	65	0.10	12.4
Curosil PFP	1.751	0.81	0.49	60	0.10	12.7
Allure-PFPP	1.650	0.72	0.58	81		
Luna Phenyl-Hexyl	1.660	0.74	0.42	57	0.09	12.2

[a] mL/column – total volume of adsorbed layer in the column calculated as a product of the surface specific adsorbed layer volume from Table 3 and mass of the base silica in the column from Table 2.

As we discussed in the Introduction section, the theoretical description of chromatographic retention process requires the definition and estimation of the stationary phase volume. Recent studies have shown that chemically bonded phase essentially could not be considered as a stationary phase since bonded chains are arranged in a primarily dense conformation ("collapsed") [27]. Current study essentially confirms that statement since there is virtually no difference in the maximum amount of either acetonitrile or methanol accumulated on the unit of surface for all different bonded phases studied. If there would be a partitioning between bonded chains we should see noticeable difference in the adsorption at least between Prodigy-3 Phenyl and Luna Phenyl-Hexyl, since their anchoring chain lengths differ significantly.

The conclusion made by Chester and Coym [33] that different solutes "see" different phase ratios, essentially emphasizes that different analytes can interact with adsorbent surface at a different distance from the surface and the surface (rather than the volume) is the retention defining parameter. On the other hand 99% of all surfaces available in HPLC column are confined in the pores of a few nm in diameter. It is logical to assume that while the analyte molecule is in the porous space inside the particle it is under the influence of the surface forces. The significance of the acetonitrile adsorbed layer volume also suggests that the total pore volume could be considered as the "stationary phase" volume. For the thermodynamic description of the chromatographic retention it is always assumed that there is an instant equilibrium of the analyte distribution between mobile and stationary phases, which is usually verified as an independence of the retention volumes on the mobile phase flow rates within a practically reasonable flow rate range. On this basis the definition of the stationary phase as the total pore volume of the adsorbent in the column and the mobile phase as the interparticle volume allows easy and independent determination of these values and the use of classical and convenient expressions (Eq. (4)) for the description of the analyte retention.

4. Conclusions

The excess adsorption isotherms of methanol, acetonitrile, and tetrahydrofuran from water were determined for phenyl-type bonded phases. The interpretation of these isotherms had shown monomolecular character of methanol adsorption and multi-layer character of acetonitrile and tetrahydrofuran adsorption from water. Accumulation of acetonitrile and THF in the porous space of used adsorbents was estimated to be over 60% of the total available pore volume in the column. This effect suggests that the analyte molecules are under the influence of the surface adsorption forces while they are anywhere in the porous space of the adsorbent, which justifies the use of the adsorbent pore volume as the volume of the stationary phase for the thermodynamic description of the chromatographic retention process.

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