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Interpretation of the excess adsorption isotherms of organic eluent components on the surface of reversed-phase adsorbents Effect on the analyte retention

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Abstract

The excess adsorption isotherms of acetonitrile, methanol and tetrahydrofuran from water on reversed-phase packings were studied, using 10 different columns packed with C_1–C_6, C_8, C_{10}, C_{12} and C_{18} monomeric phases, bonded on the same type of silica. The interpretation of isotherms on the basis of the theory of excess adsorption shows significant accumulation of the organic eluent component on the adsorbent surface on the top of ''collapsed'' bonded layer. The accumulated amount was shown to be practically independent of the length of alkyl chains bonded to the silica surface. A model that describes analyte retention on a reversed-phase column from a binary mobile phase is developed. The retention mechanism involves a combination of analyte distribution between the eluent and organic adsorbed layer, followed by analyte adsorption on the surface of the bonded phase. A general retention equation for the model is derived and methods for independent measurements of the involved parameters are suggested. The theory was tested by direct measurement of analyte retention from the eluents of varied composition and comparison of the values obtained with those theoretically calculated values. Experimental and theoretically calculated values are in good agreement.

Keywords: Adsorption isotherms; Adsorbents; Retention behavior; Henry constants; Methanol; Tetrahydrofuran; Acetonitrile; Benzene; Alkylbenzene; Toluene; Pentanone

1. Introduction

Prediction of analyte retention, optimization of chromatographic selectivity and simplification of analytical method development requires an adequate theoretical description of the reversed-phase high-performance liquid chromatography (RP-HPLC) retention process. Therefore a considerable number of publications have been devoted to the application of different retention mechanisms to the description of HPLC processes [1–25]. Eluent composition is by far the most important chromatographic parameter affecting analyte retention. This effect is usually described as a direct dependence of the analyte retention on the water–organic eluent composition pumped through the column [26–29].

Classical mathematical description of analyte retention in the partitioning model is based on the widely used relationship between the retention factor and the partition coefficient:

$$k = K\phi \quad (1)$$

where k is the analyte retention factor [$k = (V_R - V_0)/V_0$], K is the analyte partition coefficient, and ϕ is a

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phase ratio. This relationship leads to the question of the correct definition and determination of both the volume of mobile phase and the volume of stationary phase in the column [30,31]. The bonded alkyl layer of reversed-phase adsorbent is commonly assumed to be a stationary phase [26,27] and the partition coefficient is assumed to be dependent on the eluent composition [19].

The central question is: can the analyte partition into the alkyl bonded layer or is it only adsorbed on the top of this layer [32–34]. Many publications are devoted to the study of the structure, conformation, mobility and solvation of bonded alkyl chains [35–40]. It has been shown that alkyl chains bonded on silica surface have a noticeable conformational freedom [35,36,41] and certain degree of solvation [37,38], this would apparently support a partitioning model for retention [42]. However, experiments on the diffusion of fluorescence probe into the bonded layer reveal a significant increase (two orders of magnitude) of the viscosity of this layer [39] compared to the corresponding liquid alkanes. Viscosity of bonded chains increases even more with the increase of silica surface curvature. In other words, the smaller the pore diameter the more rigid the bonded chains [39].

In a previous paper [40] we measured the pore volume of different alkyl modified adsorbents using HPLC and low-temperature nitrogen adsorption (LTNA). For adsorbents modified with alkyl moieties of different chain lengths, the pore volume measured by LTNA under vacuum conditions appears to coincide with the volume measured by HPLC (less than 2% difference) [40]. The effective molecular volume of bonded chains are similar to that of corresponding liquid *n*-alkanes. These experimental results lead us to the conclusion [40] that alkyl chains bonded on silica surface are mainly in a dense, liquid-like, arrangement.

Knox and Pryde [18] proposed that the eluent in close proximity to the adsorbent surface is enriched with organic modifier. This original idea has been supported by experimental measurements of the adsorption of organic eluent components on the surface of RP packing using different methods [2–17]. The methods applied for adsorption measurements are frontal chromatography [2–8], retention of deuterated eluent components [9–13], and introduction of a minor disturbance into the equilibrated chromatographic system [9,14–17]. Kováts and coworkers [10,43,44] studied the adsorption of acetonitrile on the surface of alkyl modified adsorbents. Their interpretation of experimental results suggests a formation of an approximately 15 Å thick layer of acetonitrile on the adsorbent surface. They had shown that the thickness of this layer is almost the same for two different reversed-phase adsorbents: 3,3-dimethylbutyl-dimethylsiloxy- and ethyldimethylsiloxy-modified silicas [44]. The layer of acetonitrile described is too thick to be embedded into the relatively short chain bonded layer they used. Therefore, it was suggested that it forms an adsorbed phase of significant thickness on the top of the bonded layer.

The influence of eluent component adsorption on analyte retention, on the other hand, has not been studied extensively. In a recent review on retention mechanism in RP-HPLC by Vailaya and Horváth [45], different adsorption and partitioning models are extensively discussed. However, no discussion on the influence of the organic eluent component accumulation on the stationary phase is given.

For a binary dynamic adsorption system the relationship of the analyte retention volume, $V_R(c)$, and its adsorption, $\Gamma(c)$, is given by the following equation:

$$V_R(c) = V_0 + S \cdot \frac{d\Gamma(c)}{dc} \quad (2)$$

where V_0 is the void volume of the HPLC column, S is the adsorbent surface area. This equation is a basis for practically all methods of chromatographic adsorption measurements and was first derived by Wang et al. [3] and is rigorously discussed by Riedo and Kováts [21].

Analyte injected in the column with a binary eluent is actually the third component in the dynamic adsorption system. Correct description of the analyte retention requires the knowledge of its adsorption in a multicomponent system and the solution of the mass balance equation for that system [46].

An indication of the existence of the adsorbed phase of significant thickness [44] suggests a retention model where the analyte partitions into the adsorbed organic layer, which is on the top of the ''collapsed'' bonded phase. This model allows de-

scription of the analyte retention as a composite of its partitioning into the adsorbed organic phase followed by adsorption on the bonded layer, and requires the knowledge of the eluent component adsorption on the reversed-phase material.

In this paper, we measured the excess adsorption isotherms of three different organic eluent modifiers: acetonitrile, methanol, and tetrahydrofuran (THF) from water on alkyl-modified silica columns. These isotherms were measured using the minor disturbance method [17] on 10 silica-based adsorbents modified with alkyl ligands of different chain length. An interpretation of these isotherms and mathematical description of proposed adsorption–partitioning model are also discussed.

2. Experimental

The detailed description of used instruments, chemicals and procedures has been given in a previous publication [40]. Detailed discussion of the measurement of the system volume, column void volumes and adsorbent surface areas is also presented in the same publication [40]. Retention volumes of minor disturbance peaks used in the calculation of the excess adsorption isotherms for the dynamic binary systems that were studied are shown in the Appendix of the previous publication [40]. Three major binary systems, methanol–water; acetonitrile–water, and tetrahydrofuran–water were studied on 10 reversed-phase silica-based adsorbents modified with alkylsilanes of different chain length. The main parameters of the adsorbents are shown in Table 1.

Experiments for the measurement of gas–liquid partitioning of studied analytes were performed on an isochoric headspace system constructed in our laboratory, based on a Headspace autosampler M-150 (Asist, Cleveland, OH, USA) connected to the Hewlett-Packard gas chromatograph Model 5890 equipped with a HP-1 fused-silica capillary column.

3. Results and discussion

3.1. Excess adsorption concept and its relationship to the HPLC retention

Adsorption is an accumulation of one component in a close proximity to the adsorbent surface, under the influence of surface forces. In a liquid binary solution, this accumulation is accompanied by the corresponding displacement of another component (solvent) from the surface region into the bulk solution, thus increasing its concentration there. At equilibrium a certain amount of the solute will be accumulated on the surface in excess of its equilibrium concentration in the bulk solution, as shown in Fig. 1.

Everett [47,48] gave a strict definition for the excess adsorption value on the basis of experimentally observable quantities for binary mixtures:

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

1: Adsorbent	2: BET surface area (m^2/g)	3: Total pore volume (ml/g)	4: Mean pore diameter (Å)	5: ''Carbon'' load (C, w/w%)	6: Bonding density ($\mu mol/m^2$)
Si	374	0.965	97	0	0
C_1	292	0.804	88.6	5.03	4.16
C_2	301	0.804	88.2	6.01	3.76
C_3	295	0.781	87.1	6.73	3.38
C_4	299	0.778	86.4	7.84	3.33
C_5	288	0.746	84.8	8.3	3.03
C_6	288	0.736	83	9.26	2.99
C_8	287	0.726	81	10.4	2.72
C_{10}	264	0.687	80	11.1	2.44
C_{12}	236	0.623	78	13.4	2.61
C_{18}	182	0.531	79	17.4	2.51

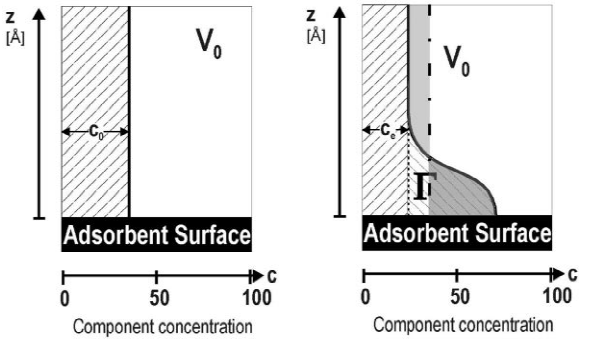

Fig. 1. Visualization of the adsorption system. The *c*-axis represents the analyte (component 2) concentration which is dependent on the distance from the adsorbent surface (*z*-axis). The system on the left represents the inactive adsorbent surface (original concentration c_0 is uniform throughout the whole volume of the liquid phase). The right system is with active adsorbent surface. c_e is the equilibrium concentration in bulk solution after adsorption. Shadow areas represent equal amounts of analyte, which was transferred on the surface from bulk solution. An excessively adsorbed amount Γ is represented by right lined area under the distribution curve.

n^0 is the analyte amount in the initial solution of mole fraction x_2^0, the mass of solid adsorbent is m its specific surface area is S, and the final equilibrium mole fraction in the liquid is x_2^e at given temperature T and pressure p. The system thus contains an amount $n^0 x_2^0$ of component 2. *If in the final state the liquid phase were of uniform composition x_2^e throughout its extent it would contain an amount $n^0 x_2^e$. This latter hypothetical state in which the composition remains uniform up to the solid surface is taken as reference state. The real system thus contains an excess of component* 2, *over and above that in the reference system, given by*:

$$n^0(x_2^0 - x_2^e) = n^0 \Delta x_2^e \tag{3}$$

and defines one measure $n_2^{\sigma(n)}$ of adsorption called the reduced surface excess of component 2 [49]. *This may be expressed in terms of the surface excess associated with unit of surface area, the areal* (*reduced*) *surface excess, $\Gamma_2^{(n)}$*:

$$\Gamma_2^{(n)} = \frac{n^0 \Delta x_2^e}{mS} \tag{4}$$

This include two assumptions:

(*i*) *Liquid is uncompressible, or molecular volumes of the solution components are constant.*

(*ii*) *Adsorbent surface is impermeable and represent a physical boundary introducing adsorption forces into the liquid phase adjacent to that surface* [47].

Assumption of a constant molar volume allows the transition from $\Gamma_2^{(n)}$ to $\Gamma_2^{(v)}$, i.e., from molar excess adsorption to volume based excess adsorption:

$$\Gamma_2^{(v)} = \frac{(c_0 - c_e)V_0}{mS} \tag{5}$$

Instead of the total number of moles of component 2, the total volume of the liquid phase in the system, V_0, and corresponding molar concentrations of component 2 before adsorption, c_0, and after the equilibrium is established, c_e, are used.

$c_0 V_0/mS$ in Eq. (5) is the total amount of preferentially adsorbed component in a binary system (shown on the left pane of Fig. 1), and $c_e V_0/mS$ is the amount left in the hypothetical equilibrated system where, according to Everett, the composition remains uniform up to the solid surface. The difference between these values would represent an excessive amount accumulated on the surface due to adsorption. This definition is shown graphically in Fig. 1.

After establishing the equilibrium there will be a certain distribution of the analyte along the *z*-axis due to the stronger attractive effect of the surface with respect to component 2. It should be noted that the profile of the analyte distribution is dependent on the types of the analyte, the solvent, the chemistry of the surface, and the initial analyte concentration. The total volume of the liquid phase will not change since the accumulation of the analyte will cause the displacement of the solvent into the bulk liquid far from the surface and constant molecular volume of all components is assumed.

For reversed-phase adsorbents, the concept of the surface area is complex [40]. The only definite surface area, which could be taken into account, is the surface area of the original silica. Any comparison of the adsorption values (usually related to the unit of the surface) should be done relative to the surface area of underlying silica.

3.2. Excess adsorption and HPLC retention

The connection of HPLC retention with adsorption phenomena is the key for the interpretation of the retention mechanism. The general concept is based on the assumption of instantaneous adsorption equilibrium in a dynamic chromatographic system and the solution of the mass-balance equation for a chromatographic column. For HPLC this was first given by Wang et al. [3] and generalized by Riedo and Kováts [21]. Both had introduced the Gibbs dividing plane prior to the application of the excess adsorption concept to the mass balance in the HPLC column.

Below we show that while using excess adsorption concept the introduction of Gibbs dividing plane is unnecessary for construction and solution of the mass-balance equation. The introduction of this plane is necessary for interpretation of the excess adsorption isotherms.

Consider an infinitely small cross-sectional layer of the column with thickness dx. F is the volumetric flow through the column and c_e is a concentration of the analyte in the bulk flow. During the time dt the amount cFdt of the analyte will move into the selected section. At the same time the amount $(c_e + \mathrm{d}c)F\mathrm{d}t$ will leave this section of the column. Total accumulation (positive or negative) in the selected cross-section of the column is $F\mathrm{d}t\mathrm{d}c$. Any accumulation in continuous media will form the gradient

$$\begin{aligned}\mathrm{grad}(c_e) &= -\left(\frac{\partial c}{\partial x}\right)_t \text{ or } F\mathrm{d}t\mathrm{d}c \\ &= -F\cdot\left(\frac{\partial c}{\partial x}\right)_t dx\mathrm{d}t \end{aligned} \quad (6)$$

The total amount of analyte in a selected cross-section of the column is distributed between the contacting phases and surfaces. If the distribution function is denoted as $\Psi(c_e)$ a general form of mass-balance equation can be written:

$$-F\cdot\left(\frac{\partial c}{\partial x}\right)_t \mathrm{d}t dx = \left[\frac{\partial}{\partial t}\Psi(c_e)\right]_x \mathrm{d}t dx \quad (7)$$

where $\Psi(c_e)$ in this equation provides a sense of the number of moles of analyte in the selected layer.

To solve this equation a specific distribution model must be assumed and an exact expression for the analyte distribution must be written. In the equilibrated column with constant flow of binary solution, some excessive amount of one component is adsorbed on the surface and the solution pumped through the column has equilibrium concentration similar to that shown in Fig. 1, right panel. The total amount of the component in the liquid phase in a small cross-section of the column can therefore be written as:

$$\Psi(c_e) = v_0 c_e + s\Gamma^{(v)}(c_e) \quad (8)$$

where v_0 is the total volume of the liquid phase in that cross-section, c_e is the component equilibrium concentration, s is the adsorbent surface area in selected cross-section, and $\Gamma^{(v)}$ is the component excess adsorption. The term $v_0 c_e$ represents the analyte amount in the whole volume, v_0, of the liquid phase of chosen cross-section, as it was defined by Everett [47]. In Eq. (8) the term $s\Gamma^{(v)}(c_e)$ represents the excessive amount adsorbed on the adsorbent surface, s.

Eq. (8) is the basis for the solution of mass-transport equation, which was discussed before [3,17,21] and leads to the following expression:

$$V_R(c_e) = V_0 + S\cdot\frac{\mathrm{d}\Gamma^{(v)}(c_e)}{\mathrm{d}c} \quad (9)$$

where V_R is the component retention volume, V_0 is the column void volume (the total volume of the liquid phase in the column), S is the adsorbent surface area, and $\mathrm{d}\Gamma/\mathrm{d}c$ is the derivative of the component excess adsorption isotherm. Parameter V_0 in this expression represents the total volume of liquid phase in the HPLC column, and S is the total surface of adsorbent in this column. Note that in this brief description, no model of adsorbed layer was introduced, nor was a position of Gibbs dividing plane defined. Use of the excess adsorption concept in HPLC does not require any additional assumptions, but the interpretation of the excess adsorption isotherms does.

Integration of the above Eq. (9) leads to Eq. (10), which we used for the calculation of the excess adsorption isotherms from the experimental data of minor disturbance peaks retention:

$$\Gamma(c_e) = \int_0^{c_e} \frac{V_R(c) - V_0}{S}\,\mathrm{d}c \quad (10)$$

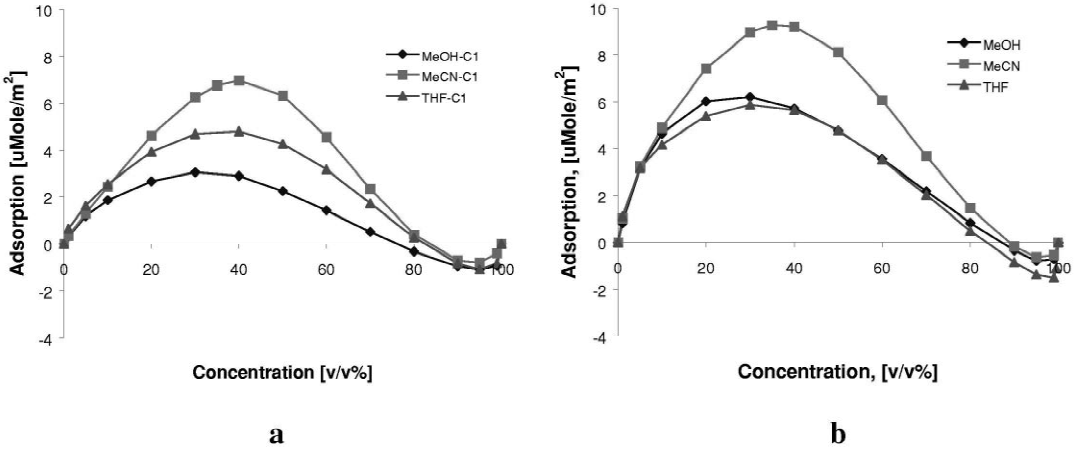

Fig. 2. Excess adsorption isotherms of acetonitrile (1); methanol (2) and tetrahydrofuran (3) from water on C_1 modified adsorbent (a) and on C_{18} modified adsorbent (b).

The resulting excess isotherms for acetonitrile, methanol and THF are shown in Fig. 2 for C_{18} type packing (a) and for C_1 type packing material (b). Full sets of measured adsorption isotherms for acetonitrile, methanol and THF on all 10 studied columns (silica modified with C_1–C_{18} alkyl chains) are shown in Figs. 3–5, respectively.

As seen in Fig. 2 excess adsorption isotherms for acetonitrile are the highest on both adsorbents (C_1 and C_{18}) and the adsorption isotherm of THF practically coincides with that for methanol on the C_{18} surface. Although the excess amounts of tetrahydrofuran and methanol adsorbed on the surface are the same, the molecular volumes of these two

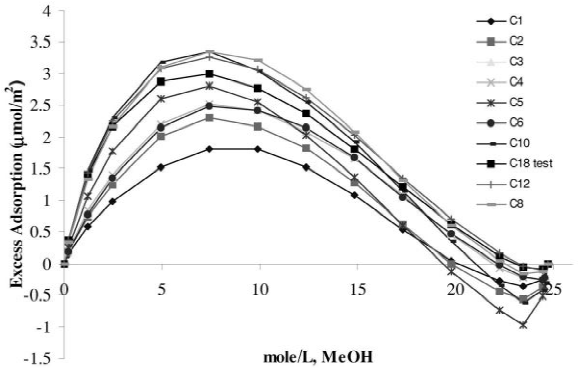

Fig. 4. Methanol excess adsorption isotherms on C_1, C_2, C_3, C_4, C_5, C_6, C_8, C_{10}, C_{12} and C_{18} modified adsorbents.

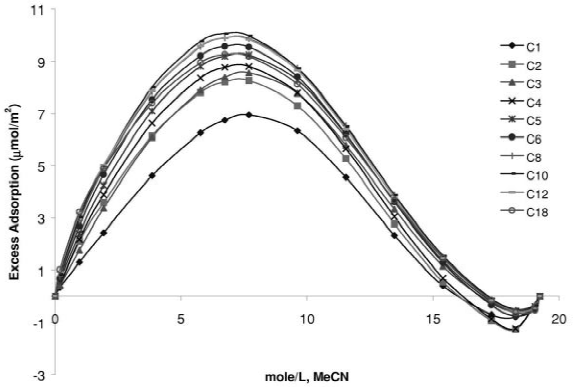

Fig. 3. Acetonitrile excess adsorption isotherms on C_1, C_2, C_3, C_4, C_5, C_6, C_8, C_{10}, C_{12} and C_{18} modified adsorbents.

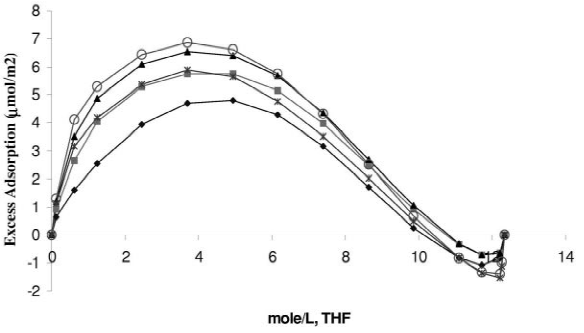

Fig. 5. THF excess adsorption isotherms on C_1, C_2, C_3, C_4, C_5, C_6, C_8, C_{10}, C_{12} and C_{18} modified adsorbents.

compounds are different (67 $Å^3$ for methanol and 135 $Å^3$ for THF).

Overlay of the excess adsorption isotherms of the same components on different surfaces (Figs. 3–5) reveals the similarity of their adsorption behavior. All of the isotherms show slight negative excess at high organic concentration. This indicates a preferential adsorption of water and is an indication of the presence of accessible residual silanols. At the region between 8 and 17 mol/l of acetonitrile (Fig. 3) all of the isotherms have negative slope. This region represents maximum filling of available hydrophobic adsorbent surface with acetonitrile. Essentially this means the formation of an adsorbed layer composed of pure acetonitrile.

The excess amount adsorbed, Γ, is known, but the amount, a_e, which was already on the surface from the equilibrium solution (only the concentration, c_e) is not known. A model of the adsorbed layer structure is needed for the estimation of that amount. The Gibbs dividing plane separates the adsorbed layer from the bulk solution and the uniformity of the analyte concentration throughout the volume of each of these phases is assumed. Measurement and calculation of the excess adsorption is therefore possible without assumption of any specific adsorption model, however, the interpretation of the adsorption isotherm requires the introduction of a model.

The existence of the hypothetical plane parallel to the adsorbent surface with distance τ from it will be assumed. Above this plane, component concentrations are equal to the equilibrium concentration. Between this plane and the adsorbent surface, the amount of preferentially adsorbed component per unit of surface area may be expressed as follows:

$$n_{ads} = c_e\tau + \Gamma(c_e) \quad (11)$$

where n_{ads} is the total amount adsorbed in mol/m^2, c_e is the equilibrium concentration in mol/ml, τ is the adsorbed layer thickness in Å multiplied by 10^{-4} for unit conversion, and $\Gamma^{(v)}(c_e)$ is the excess adsorption in mol/m^2.

Linear decrease of $\Gamma^{(v)}(c_e)$ values with the increase of the component concentration in the region between 7 and 18 mol/l indicates complete filling of the adsorbed layer and n_{ads} is constant in this region. Total amount adsorbed in the finite thickness layer could be found from Eq. (11) by extrapolation of the slope of the excess adsorption isotherm in linear region to the intercept with the y-axis, where $c_e = 0$ and $n_{ads} = \Gamma(0)_{extrapolated}$ (according to the method suggested by Everett [47]). Estimation of the maximum adsorbed amount from the excess adsorption isotherm is shown in Fig. 6.

Since the maximum adsorbed amount represents a complete filling of the adsorbed layer with the corresponding component, and since a constant molar volume in the adsorbed and bulk phases is assumed, the volume of adsorbed layer per unit of surface area, v_{ads} can be calculated as:

$$v_{ads} = n_{max}v_{mol} \quad (12)$$

where v_{mol} is the adsorbed component molar volume. The values for the volume of adsorbed phase for all studied systems are shown in Table 2. Value of v_{ads} has a dimension of ml/m^2, which could be transferred into linear dimensions representing the apparent thickness of this adsorbed layer. The thickness of the adsorbed layer for all measured isotherms on all studied adsorbents was calculated. The dependencies of that thickness on the number of carbons of alkyl chains bonded on silica surface are shown in Fig. 7.

Methanol forms an adsorbed layer of approximately 2.5 Å thickness, which clearly indicates the

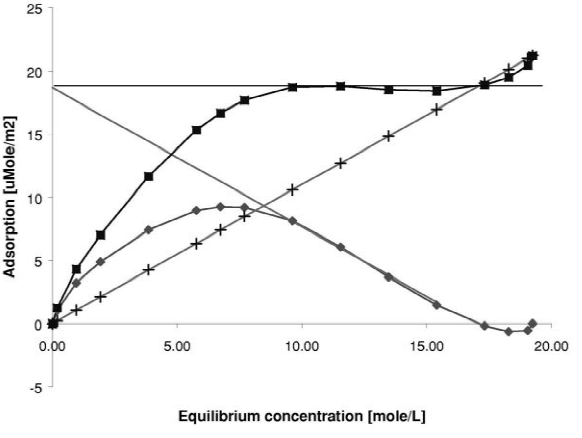

Fig. 6. Estimation of the maximum adsorbed amount on the basis of the model of finite thickness adsorbed layer. Diamonds are the experimental excess adsorption isotherm, crosses are the amount of the adsorbed component in the adsorbed layer from equilibrium concentration, and squares are the total amount of analyte in the adsorbed layer.

Table 2
Corrected pore volume and the volume of bonded phase

1: Carbon number	2: ''Free'' volume between bonded chains ($\mu l/m^2$)	Adsorbed layer volume		
		3: MeCN ($\mu l/m^2$)	4: THF ($\mu l/m^2$)	5: MeOH ($\mu l/m^2$)
C_1	0	0.85	0.90	0.163
C_2	0.084	0.98	1.00	0.200
C_3	0.178	0.99		0.203
C_4	0.239	1.03	1.09	0.198
C_5	0.335	1.04		0.229
C_6	0.395	1.06		0.247
C_8	0.546	1.09	1.16	0.258
C_{10}	0.704	1.09		0.255
C_{12}	0.75	1.08		0.237
C_{18}	0.957	1.01	0.98	0.215

monomolecular character of its adsorption on the surface of the reversed-phase adsorbent. On the other hand, the corresponding thickness for acetonitrile and tetrahydrofuran coincide at about 14 Å. This indicates that these components form multilayer adsorbed phases.

3.3. ''Free'' volume of the bonded phase

According to Berendsen and DeGalen [49], C_{18} chains in an all-*trans* conformation have the length, *l*, of 25 Å, molecular volume, $v_{C_{18}}$, of 500 $Å^3$ [40], and bonding density, d_b, of 2.52 $\mu mol/m^2$. In a previous paper [40] the concept of ''free volume'' – the space available for solvent or any other molecules to penetrate between bonded chains was discussed. Comparison of that ''free'' volume [40] with the volume of the adsorbed organic eluent component is shown in the Table 2. A graphical representation of the adsorbed layer volume and a ''free'' volume between bonded chains is given in Fig. 8. The adsorbed layer volume is much less dependent (essentially independent) on the number of carbons in the bonded ligands than is the ''free'' volume. Acetonitrile and tetrahydrofuran have an adsorbed layer volume more than four-times higher than methanol.

The amount of adsorbed methanol is smaller than

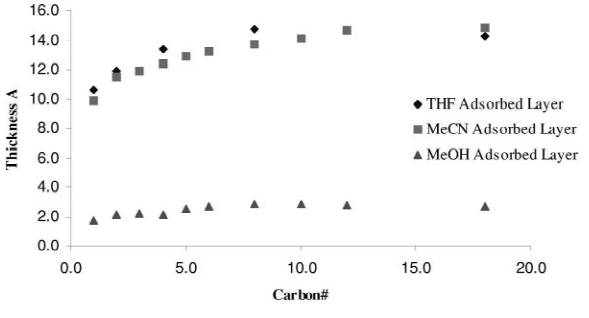

Fig. 7. Adsorbed layer thickness for THF, acetonitrile, and methanol on the surface of silica-based adsorbents modified with alkylsilanes of different chain length.

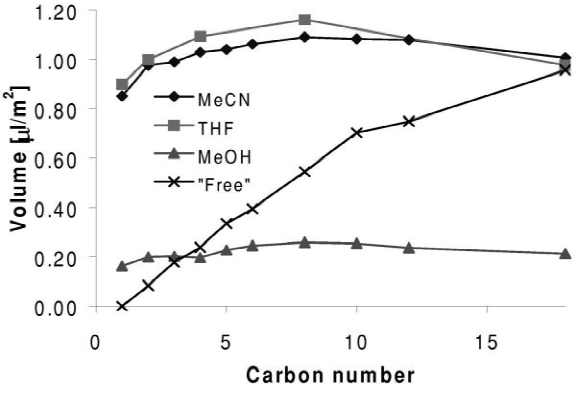

Fig. 8. Volume of adsorbed organic layer of acetonitrile, methanol, and tetrahydrofuran on adsorbents with different bonded chains, and ''free'' volume between bonded chains (in all-*trans* conformation).

the volume available between bonded chains (Fig. 8) for most of the phases studied. If methanol had solvated these chains, an increase of the adsorbed amount with the increase of the bonded chain length would be expected, but the amount of adsorbed methanol remained constant.

The volume of adsorbed acetonitrile and THF is significantly higher than the ''free'' volume available for all adsorbents except C_{18}. As the length of the bonded ligands increases, the ''free'' volume between those chains also increases. If acetonitrile or THF penetrated the ''free'' volume between bonded chains, an increase of length of these chains would have led to the corresponding increase of the total amount of acetonitrile accumulated on the surface. However, with the increase of the length of bonded chains the volume of adsorbed acetonitrile does not increase.

On all studied adsorbents from C_1 to C_{18}, the volume of the adsorbed acetonitrile remains fairly constant. If C_1 and C_2 type adsorbents have enough surface energy to accumulate up to 1 $\mu l/m^2$ of acetonitrile on the top of their surface, it would be logical to assume that more hydrophobic adsorbents (C_8, C_{12}, C_{18}) would be able to do the same. Consequently if there is a penetration of acetonitrile molecules between bonded chains, the total amount of accumulated acetonitrile would be the sum of the amount adsorbed on the top plus the amount accumulated between chains. This would result in almost 2 $\mu l/m^2$ of acetonitrile accumulated on the C_{18} surface. Experimental data show that this is not occurring. The volume of the adsorbed layer is almost independent of the chain length. This means that it is also independent of the intermolecular free volume in the bonded layer. Therefore, all adsorbed acetonitrile is accumulated on the top of the bonded layer, leading to the constant acetonitrile volume adsorbed on all studied surfaces.

In this case, the ''free'' volume inside the bonded layer comprised of flexible chains is unoccupied, this means that it is actually nonexistent. Long alkyl chains are self-associated (''collapsed'') with dispersive forces between adjacent bonded chains being stronger than their possible solvation with polar acetonitrile molecules. This conclusion is supported by a study of the geometry of modified adsorbents [40]. It was shown that the volume of the bonded layer (determined as the difference between the pore volume of bare silica and alkyl-modified silica) is equal to the corresponding volume of liquid alkanes.

All three studied adsorbates (acetonitrile, methanol and tetrahydrofuran) show practical independence of the amount adsorbed on the length of the bonded chains. THF and acetonitrile both form an adsorbed layer of significant thickness (up to 14 Å). Methanol, on the other hand, forms only a monomolecular layer of 2.5 Å thickness. The obtained parameters of the adsorbed layer thickness are in the excellent agreement with those calculated by Ha et al. [44] from excess adsorption isotherms of acetonitrile from water and THF from water on completely different types of reversed-phase adsorbents. The dependence of the thickness of the collapsed bonded layer and corresponding adsorbed organic layer on the number of carbon atoms of bonded chains is shown in Fig. 9.

3.4. Adsorption–partitioning retention model

The existence of an adsorbed layer of considerable thickness with a composition different from that of the mobile phase suggests the need for a model for the description of the analyte retention from a binary mobile phase.

The following assumptions are made: (i) binary mobile phase is pumped through the column at a constant composition long enough to establish

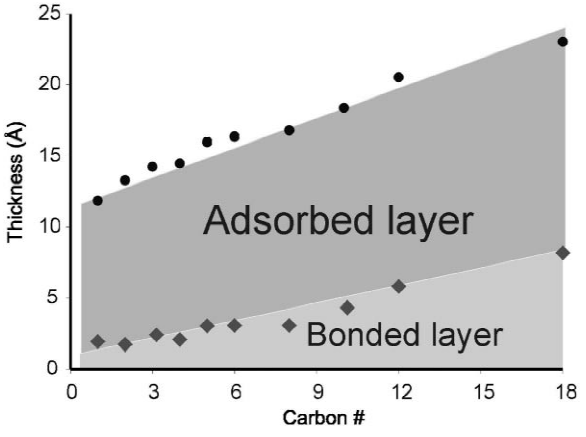

Fig. 9. The dependence of the acetonitrile adsorbed layer and collapsed bonded layer thicknesses on the length of the bonded chains.

equilibrium and to form a stable adsorbed organic layer; (ii) analyte is injected on the column in a small volume of a dilute solution; (iii) a small amount of analyte does not disturb the equilibrium of the binary eluent in the column.

Analyte retention in that system could therefore be described as superposition of two processes: analyte partitioning from the eluent into the adsorbed layer followed by its adsorption from that layer on the surface of the bonded phase.

The analyte distribution function in the small column cross-section would be:

$$\Psi(c_e) = v_m c_e + v_s c_s + s\Gamma(c_s) \quad (13)$$

where v_m is the volume of the equilibrium liquid phase in the selected cross-section of the column with thickness d_x, c_e is the analyte equilibrium concentration in the mobile phase, v_s is the volume of the adsorbed phase, c_s is the analyte concentration in that phase at the equilibrium with the mobile phase and with the surface, s is the adsorbent surface area, and Γ is the excess adsorption of the analyte on the surface from the stationary phase (where the analyte concentration is c_s).

The analyte distribution between two liquid phases (eluent and adsorbed phase) at equilibrium could be described as follows:

$$c_s = K_p c_e \quad (14)$$

where K_p is the analyte distribution constant between eluent and adsorbed phase. Since the analyte is injected in very low amount, its adsorption on the surface of bonded phase is assumed to be in the Henry region. The adsorption process also could be described as:

$$\Gamma(c_s) = K_H c_s \quad (15)$$

or substituting from Eq. (14):

$$\Gamma(c_s) = K_H K_p c_e \quad (16)$$

where K_H is a Henry adsorption constant.

The final form of the distribution function, accounting that $v_0 = v_m + v_s$, therefore will be:

$$\Psi(c_e) = [v_0 + (K_p - 1)v_s + sK_H K_p]c_e \quad (17)$$

Applying this function into the mass-balance Eq. (7) and integrating it, the final equation for the analyte retention in binary eluent is obtained. In the expression below, only the analyte distribution coefficient is dependent on the eluent composition:

$$V_R(c_{el}) = V_0 - V_s + K_p(c_{el})[V_s + SK_H] \quad (18)$$

$V_R(c_{el})$ is the analyte retention as a function of the eluent concentration, V_0 is the total volume of the liquid phase in the column, V_s is the volume of adsorbed layer, $K_p(c_{el})$ is the distribution coefficient of the analyte between the eluent and adsorbed phase, S is the adsorbent surface area, and K_H is the analyte Henry constant for its adsorption from pure organic eluent component (adsorbed layer) on the surface of the bonded phase.

In this case, the retention parameter is no longer proportional to any thermodynamic constant, but it is a superposition of several thermodynamic parameters, each of them related to a different process.

Since all involved parameters can be measured independently, this model can be experimentally verified. Void volume and adsorbent surface measurements has been discussed in our previous publication [40]. Volume of the adsorbed layer is a product of the adsorbed layer thickness and adsorbent surface area. The Henry constant of the analyte is the slope of its adsorption isotherm from pure organic component. This corresponds to a binary system and can be calculated using Eq. (9) from the data on analyte retention from pure organic (single component) eluent, $V_R(100)$:

$$K_H = \frac{V_R(100) - V_0}{S} \quad (19)$$

The only parameter dependent on the eluent composition is the analyte distribution constant, which represents the analyte distribution between two hypothetical and practically miscible phases (mobile phase and adsorbed organic layer). The distribution constant of any component between the liquid and gas phases is significantly dependent on the liquid phase composition. The gas–liquid equilibrium distribution constant is an energetic parameter; its logarithm represents the difference of the analyte free Gibbs energy between two contacting phases. Consequently the analyte distribution constants between the eluent and the gas phase and between the liquid, which composition is equivalent

to that of the adsorbed phase, and a gas phase will be different. The logarithm of the ratio of these two constants will represent the energetic difference of the analyte transfer between these two liquid phases.

It is possible to measure the analyte distribution between these phases independently. If ideal analyte behavior in the vapor phase is assumed, the analyte distribution between the solution and vapor phase over it can be measured using headspace method:

$$K_1 = \frac{c_{\text{eluent}}}{c_{\text{vapor}}}, \quad K_2 = \frac{c_{\text{organic}}}{c_{\text{vapor}}},$$

$$K_p(c_{el}) = \frac{K_2}{K_1} = \frac{c_{\text{organic}}}{c_{\text{eluent}}} \tag{20}$$

Measurement of the analyte distribution constants between the liquid and vapor phases is a challenge. We have used variable volume headspace method [50–52] for the determination of above mentioned distribution constants for alkylbenzenes.

These constants for the alkylbenzenes homologous series from benzene to amylbenzene were obtained for their solutions in acetonitrile–water mixtures in the 100:0 to 60:40 region.

Calculated analyte distribution coefficients (Table 3) increase with the decrease of the concentration of acetonitrile in the eluent, reflecting the increase of the energetic preference for the analyte molecule to penetrate into the layer of adsorbed acetonitrile and be retained. According to Eq. (18) $K_p(c_{el})$ is the only parameter defining the analyte retention dependence on the eluent composition. Retention volumes for studied analytes calculated using Eq. (18) from data in Table 3 and experimental retention volume for the same analytes are shown in Fig. 10.

The proposed model was used for the prediction of the HPLC retention of polar component, *n*-butanone, on C_8, C_{12} and C_{18} reversed-phase columns. Distribution constants for *n*-butanone are shown in Table 4; corresponding Henry constants are shown in Table 5. Comparison of the predicted and experimental retention factors is shown in Fig. 11.

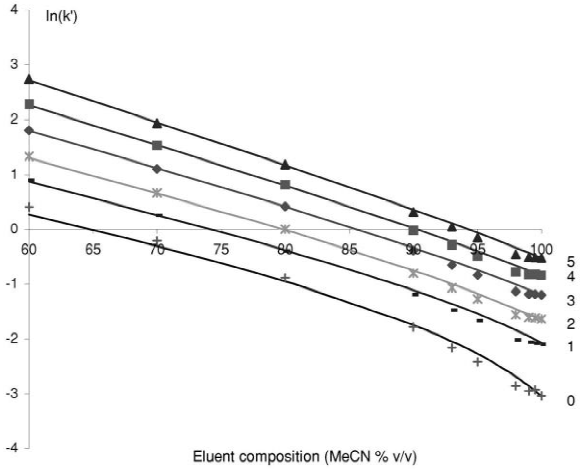

Fig. 10. Correlation of the alkylbenzene retention factors calculated from experimental data (dots) with those obtained from adsorption–partitioning model (lines). Void volume for Luna-C_{18} column was measured using minor disturbance method [31]. Dependencies are marked on the right according the number of carbons in the alkyl chain (from 0 for benzene, to 5 for amylbenzene).

Reasonable agreement is obtained for the *n*-butanone retention on columns with different bonded phases. Although independent measurement of the analyte distribution coefficients between eluent and adsorbed organic layer is tedious and time consuming, the good correlation between theoretical and experimental retention values for this polar compound confirms the validity of the proposed retention model.

Table 3
Calculated analyte distribution coefficients between pure acetonitrile and different composition of acetonitrile–water mixtures and Henry constants

Analyte	Acetonitrile–water composition (v/v%, acetonitrile)							K_H [a]
	60	70	80	90	95	99	100	
Benzene	7.61	4.58	2.76	1.66	1.29	1.052	1.000	0.455
Toluene	9.30	5.33	3.05	1.75	1.32	1.057	1.000	1.104
Ethylbenzene	11.37	6.19	3.37	1.84	1.36	1.063	1.000	1.623
Propylbenzene	13.90	7.20	3.73	1.93	1.39	1.068	1.000	2.532

[a] K_H is the analyte Henry constant.

Table 4
n-Butanone liquid–vapor distribution constants (headspace measurements) and calculated distribution coefficients between acetonitrile and acetonitrile–water mixtures of different composition

Composition (%, v/v, acetonitrile)	Distribution constant (liquid–vapor)	Partitioning coefficient
100	3079	1.000
90	2964	1.039
80	2123	1.450
70	1631	1.888
60	1348	2.283
50	884.2	3.483
40	588.7	5.231
30	354.9	8.677
20	253.4	12.15
10	195.5	15.75
0	182.1	16.91

4. Conclusion

The analysis of the experimental excess adsorption isotherms has shown the formation of the adsorbed layer of organic eluent component primarily on the top of the surface of alkyl chains bonded on silica surface. This confirms the conclusion of our previous publication [40] that bonded alkyl chains are mainly in the self-associated (collapsed) state.

Acetonitrile and tetrahydrofuran form thick adsorbed layers on the hydrophobic surface of reversed-phase adsorbents. This layer is equivalent to four or five molecular layers stacked on the top of each other. Methanol, on the other hand, forms only a monomolecular adsorbed layer on the same surface. The explanation of this effect requires further investigation, and may be based on the self-association of acetonitrile and tetrahydrofuran on the hydrophobic surface.

The thickness of that adsorbed layer is independent on the length of underlying bonded alkyl chains. This suggests the prevalent character of dispersive forces in adsorption interactions of the organic eluent components.

Table 5
n-Butanone Henry constants for used adsorbents

	Adsorbent		
	C_{18}	C_{12}	C_8
Henry constant (ml/m^2)	0.182	0.130	0.102

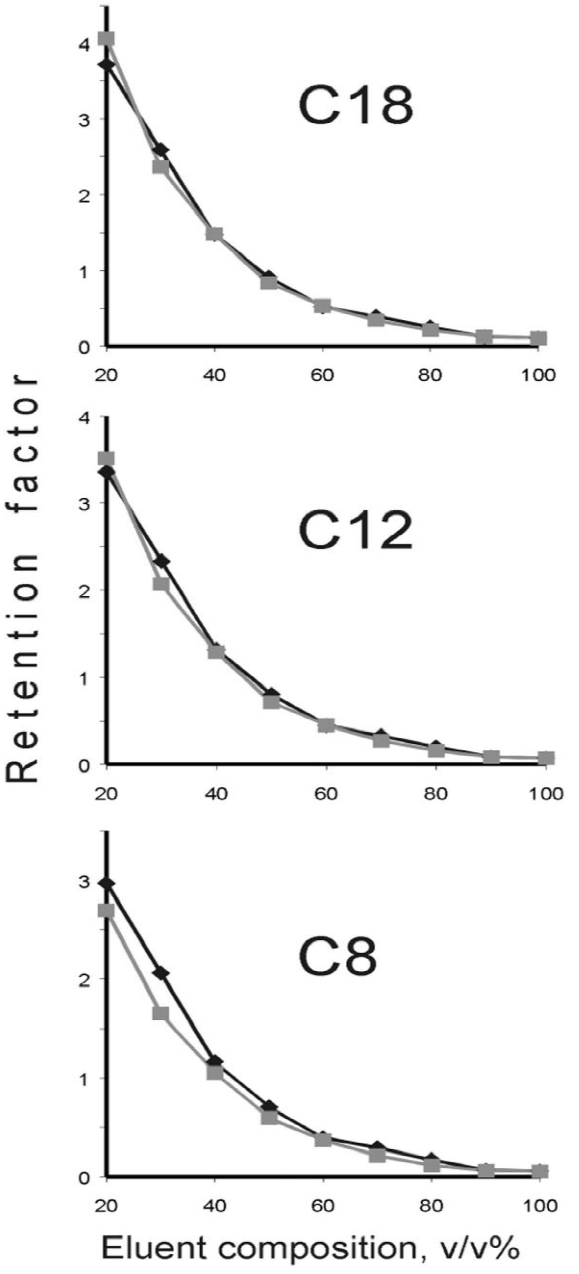

Fig. 11. Comparison of experimental and predicted retention factors for *n*-butanone on different columns. Squares are predicted values; rhombs are experimental data.

A model of adsorption–partitioning retention model is suggested. Analytical solution of the mass-balance equation describing the analyte retention in

the chromatographic system with binary eluent is obtained. Practical applicability of proposed model is demonstrated for polar and nonpolar analytes.

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