Effect of polar interactions on the nonlinear behavior of phenol and aniline in reversed phase liquid chromatography

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ABSTRACT

The effect of the coverage density and the activity of the unreacted silanol groups of the non-endcapped octadecyl bonded phase on the adsorption properties were investigated. The adsorption of two polar low molecular weight compounds with weak acidic (phenol) and with basic (aniline) character was measured. Adsorption data were acquired by frontal analysis from methanol–water and acetonitrile–water solutions to investigate the influence of the type of the organic modifier on the solute retention mechanism and adsorption under non-linear conditions. The adsorption behavior of phenol changes from Langmuir type (from aqueous mixture of methanol) to BET type (from aqueous mixture of acetonitrile). The adsorption of aniline becomes BET-type, regardless of the mobile phase composition in the tested range of the surface coverage of octadecyl ligands. The nature of the organic modifier significantly affects the retention mechanism and the shape of the overloaded elution bands of the studied compounds.

1. Introduction

The separation of compounds in reversed phase liquid chromatography (RPLC) results from the difference of their distribution between the mobile phase (usually aqueous solution of an organic solvent) and the hydrophobic bonded layer. Solute molecules may adsorb on the surface of the stationary bonded phase. An alternative mechanism of the distribution is the partition of the analyte between the mobile and bonded phases [1,2].

The separation selectivity in reversed-phase chromatography using octadecyl (C_{18}) bonded phase is optimized by the quantitative and qualitative composition of the binary mobile phase [3]. When the organic solvent in the mobile phase is replaced by another one, the properties of the stationary phase also change. The changes of the separation selectivity may be caused by solute interaction with the solvated stationary phase (organic ligands and solvent molecules are extracted into the hydrophobic region of bonded ligands) [4–7]. The quantitative mobile phase composition also has an important effect on the values of all the parameters of the adsorption isotherm and on the retention factor of the solute [1]. The changes of the mobile phase composition affect significantly the properties of the interfacial region the C_{18}-bonded silica surface

[8–11]. It is caused by two effects. First, the increase of the organic solvent concentration in the aqueous mobile phase increases the competition for the hydrophobic adsorption sites between the solute and organic modifier. That results in the decrease of the retention factor of the analyte when the concentration of the organic modifier in the mobile phase increases. Second, the structure of the C_{18} chains that define the interphase between the mobile phase and the solid silica is affected because of the preferential adsorption of water and organic modifier on the residual accessible silanols and organic ligands [3,12–14]. At low organic modifier content in the mobile phase, the bonded chains are brought together by the intermolecular C_{18} dispersive interactions while they tend to exclude water. At high organic modifier concentration, the rupture of the octadecyl intermolecular interactions via chain solvation by the modifier and the formation of a 'brush-like' structure is observed [3,14]. The solvation process depends on the type of organic modifier, the length of organic ligands and their coverage density [14–17].

In a previous work [18] we have examined the effect of the nature of the organic solvent on the nonlinear adsorption behavior of phenol on endcapped reversed phase packing materials with different surface coverage of the octadecyl bonded ligands. The endcapping process shields a significant amount of the unreacted residual silanol-groups and make the hydrophobic interactions predominant even at low surface coverage of the C_{18} ligands on the retention process of phenol molecules.

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The goal of our present work was to investigate the effect of the significant amount of polar groups results the lack of the end-capping on the nonlinear retention behavior of two polar test compounds with low molecular weight. The Galushko test [19] is a well-known method to describe the silanol activity of a modified silica column using data of the retention difference between phenol and aniline molecules from the same mobile phase under linear condition. For that reason, we have chosen aniline and phenol as our test compounds to compare their retention behavior under nonlinear conditions.

We give a comprehensive overview of the effects that influence the overloaded elution band shapes under these conditions in reversed phase liquid chromatography. By means of five in-house-made non-endcapped octadecyl bonded phases with different surface coverage, the effect of the residual polar groups, and the change of the surface hydrophobicity was tracked by monitoring the change of the overloaded elution bands of the test molecules.

The overloaded elution bands of phenol and aniline were modeled on the basis of their adsorption isotherm data, the connection between the change of the isotherm parameters with the change of the surface coverage was determined. Furthermore, a numerical method was used to predict the overloaded band shape on columns with any desired surface coverage.

2. Theory

2.1. Frontal analysis for isotherm determination

Frontal analysis is widely used and the most accurate method to characterize the physico-chemical properties of chromatographic adsorbent beds. Two main variations of the method exist to perform frontal analysis in liquid chromatography: the single step, and the stepwise technique. Detailed methodology of the methods can be found in the literature [20–22]. Since the two techniques are equivalent, to save chemicals and to avoid the error results from the re-equilibration steps with the pure mobile phase between each breakthrough curve, we applied the stepwise method in our work. The principle of the stepwise technique is to perform consecutive and abrupt concentration increases of the compound studied in the mobile phase pumped into the column. After the concentration change in the mobile phase, the column has to be washed with the new eluent for sufficient time to reach the equilibrium between the mobile and the stationary phase. The equilibrium is indicated by the formation of a plateau on the frontal chromatogram between two breakthrough fronts. The calculation of the solute concentration on the stationary phase q at a given mobile phase concentration C, in the case of the stepwise method is given by the following equation [23]:

$$q_{i+1} = q_i + \frac{(C_{i+1} - C_i)(V_{F,i+1} - V_0)}{V_a} \tag{1}$$

where q_i and q_{i+1} are the adsorbed concentration of the analyte when the stationary phase is in equilibrium with the solute mobile phase concentrations C_i and C_{i+1} at the ith and $(i+1)$th step, respectively. If the observed fronts are symmetrical with sharp boundaries $V_{F,i+1}$, the retention volume of the front can be calculated at the volume corresponding to the maximum numerical value of the first derivative of the breakthrough curve of the chromatogram (the inflexion point) at the $(i+1)$th step, V_0 is the void volume of the system include all the volumes contribute in the band broadening, and V_a is the volume of the adsorbent.

2.2. Isotherm models

The Langmuir isotherm [24] describes ideal adsorption on homogeneous surfaces.

$$q = \frac{aC}{1 + b_s C} = \frac{q_s b_s C}{1 + b_s C} \tag{2}$$

where a is the Henry constant of the adsorption – the initial slope of the isotherm – given by

$$a = q_s b_s \tag{3}$$

where q_s is the mono-layer saturation capacity of the adsorbent, and b_s is the related adsorption equilibrium constant.

In several cases, the adsorption of the analyte is not ideal. The interaction between the adsorbed molecules is significant, and multilayer adsorption takes place. Brunauer, Emett, and Teller described a widely used model for non-ideal adsorption on homogeneous surfaces [25]. The equation extended to liquid–solid equilibria is written as:

$$q = \frac{q_s b_s C}{(1 - b_L C)(1 - b_L C + b_s C)} \tag{4}$$

where q_s is the monolayer saturation capacity, with the equilibrium constants for the adsorption on the surface b_s, and b_L is the equilibrium constants of the interaction between the adsorbed molecules. If the interaction between analyte molecules is strong, b_L becomes larger; if there is no interaction between adsorbed molecules, $b_L = 0$ and the equation reduces to a simple Langmuir equation.

2.3. Equilibrium dispersive model of chromatography

The equilibrium dispersive model of chromatography assumes constant equilibrium between the stationary and mobile phases, and uses an apparent dispersion term to account for the band broadening effects. The mass balance equation of the chromatographic system can be written as [20]:

$$\frac{\partial C}{\partial t} + F\frac{\partial q}{\partial t} + u\frac{\partial C}{\partial z} = D_a \frac{\partial^2 C}{\partial z^2} \tag{5}$$

where z is the length, t the time, u the mobile phase linear velocity, F is the phase ratio. When the molecular weight of the studied compound is small and its polarity is moderate, an instantaneous equilibrium between the stationary and the mobile phases can be assumed and the above model can be used with good accuracy. The apparent dispersion coefficient D_a defined as:

$$D_a = \frac{Hu}{2} \tag{6}$$

where H is the height equivalent to a theoretical plate. The isotherm parameters derived from the frontal analysis were used to predict overloaded elution band of the given compound. The overloaded elution bands were modeled using the Martin–Synge algorithm. Detailed method description can be found elsewhere [26].

3. Experimental

3.1. Instruments

The frontal analysis and overloaded band profile measurements were all acquired using an Agilent (Palo Alto, CA, USA) 1100 liquid chromatograph. This instrument includes a binary solvent delivery system, an auto-sampler with a 100 μL sample loop, a diode-array UV-detector, a column thermostat and a data acquisition station.

Table 1
Main properties of the five in-house-made non-endcapped reversed phase C_{18} columns.

$\alpha_{C_{18}}$ (μmol/m^2)	V_G (cm^3)	V_m[a] (cm^3)	V_a[a] (cm^3)	V_p[a] (cm^3)	ϵ_T	S_p[b] (m^2/g)
3.27	2.076	1.247	0.829	0.446	0.601	203.0
2.95	2.076	1.251	0.826	0.473	0.603	220.3
1.68	2.076	1.437	0.639	0.618	0.692	270.0
1.11	2.076	1.547	0.529	0.697	0.745	305.6
0.33	2.076	1.711	0.365	0.833	0.824	281.1

[a] Based on ISEC measurements with polystyrene standards dissolved in pure THF.
[b] Derived from LTNA measurements.

3.2. Materials and reagents

Five chromatographic columns with different surface coverage were studied. All the columns were prepared by the modification of the same batch of 5 μm average particle diameter and 100 Å average pore diameter Kromasil 100 silica gel (Akzo Nobel, Bohus, Sweden). All the adsorbents were packed into stainless steel tubes with uniform dimensions (4.6 mm × 125 mm). The column packings and columns were characterized using inverse size-exclusion chromatography and low temperature nitrogen adsorption. The detailed description of the neat silica and the methods used for the characterization can be found elsewhere [27]. The main physicochemical properties are listed in Table 1. All solvents were of HPLC isocratic grade, purchased from Scharlau Chemie S.A. (Barcelona, Spain). Water was purified using Milli-Q system (Millipore, El Paso, TX, USA). All eluents were degassed in ultrasonic bath under vacuum. Phenol and aniline were purchased from Sigma–Aldrich (Steinheim, Germany).

3.3. The practical execution of frontal analysis, and measurements of overloaded elution bands

The frontal analysis experiments were measured using pump A to deliver the pure mobile phase 30% organic modifier (methanol or acetonitrile):70% water mixture. Pump B was used to deliver the solutions of aniline and phenol dissolved in the same mobile phase. The applied mobile phase compositions were chosen to ensure enough retention (retention factor larger than $k' = 1$) on columns with lower surface coverage to perform the frontal measurements. On the other hand, the retention factor of phenol and aniline should avoid extremely high values on columns with high surface coverage. The content of organic modifiers in the mobile phases results from this compromise. From methanol–water mixtures, both test molecules on all the five columns were measured. From acetonitrile–water mixture, on the column with the lowest surface coverage the isotherm of the test molecules cannot be measured due to their low retention factor. To ensure sufficiently large retention factor on this column from acetonitrile–water mixture, with the decrease of the organic content of the mobile phases would result unmanageable high retention factor from methanol–water mobile phases on columns with high surface coverage. The maximum mobile phase concentrations of the studied compounds were chosen according their solubility in the applied hydro-organic mixtures. The maximum concentration of the phenol stock solution was 40 g/dm^3 in both mobile phases, the concentration of aniline was 40 g/dm^3 in methanol–water and 33 g/dm^3 in acetonitrile–water mixture, because of its lower solubility in the acetonitrile–water mixture.

During the frontal analysis experiments, the pumps performed twenty-five consecutive identical concentration steps between B% of 0–100%, with 5% (v/v) increase of the stock solution of the analyte in the total flow of the mobile phase. In the case of measuring the overloaded elution bands, pump B was used to inject 2.00, 1.50, 1.00 and 0.50 cm^3 of the solutions into the pure mobile phase, and the injected bands were eluted by pumping the pure mobile phase.

The UV detection was carried out not on the adsorption maximum of the studied compounds, but at $\lambda = 293$ nm to avoid the strong non-linear response of the detector (absorbance above 1500 mAU), due to the large analyte concentration. The experiments were carried out at constant 1.0 cm^3/min flow rate and at 298 K.

4. Results and discussion

4.1. Determination of the adsorption isotherms on columns with different surface coverage

Fig. 1 illustrates the consecutive breakthrough curves of phenol and aniline and the corresponding retention volume delays between two consecutive breakthrough fronts. The series of breakthrough curves measured from methanol–water mixture on high surface coverage (3.27 μmol/m^2), and from acetonitrile–water mixture on column with low surface coverage (1.11 μmol/m^2) represent the two endpoints of our experiments. On high surface coverage, using weaker eluent in the case of phenol, the retention volume delays show a uniform decreasing trend, which suggests Langmuir-type adsorption behavior. In the case of aniline, using acetonitrile–water as mobile phase, the same graph shows different characteristics. At low concentrations of aniline, the retention volumes show slightly decreasing trend, but with the increase of the analyte concentration, the retention volume delays start to increase. This suggests an opposite, anti-Langmuirian behavior. In the following parts we will discuss in detail this change in the adsorption processes.

Since the breakthrough curves were symmetrical with sharp boundaries, the isotherm data points were calculated in all experimental conditions according to Eq. (1). To choose the proper adsorption isotherm equation, non-linear regression was performed and the equation with the smaller residual sum of squares (RSS) was used to model the given adsorption behavior of the low molecular weight compound. The numerical values of the isotherm parameters were determined using the Levenberg–Marquardt algorithm; the iterations were repeated with different initial values to avoid finding a local minimum of the set of the parameters. Fig. 2 shows the raw adsorption isotherm data points and the best fitted isotherm equations of phenol and aniline from methanol–water and acetonitrile–water solutions. The numerical values of the fitted parameters are listed in Table 2.

4.1.1. Adsorption of phenol

4.1.1.1. MeOH–H$_2$O mobile phase. The adsorption isotherms of phenol from methanol–water solutions were uniformly convex upward in the whole concentration range. That shape corresponds to type I of the isotherm classification of Brunauer et al. [28].

The type I adsorption isotherms were modeled using the simple Langmuir and the bi-Langmuir equations. At higher surface coverage of the octadecyl ligands, the bi-Langmuir equation – which is often used in the literature to model the adsorption behavior of phenol from methanol–water mixtures – gives smaller RSS value. On the other hand, at low surface coverage, the simple Langmuir
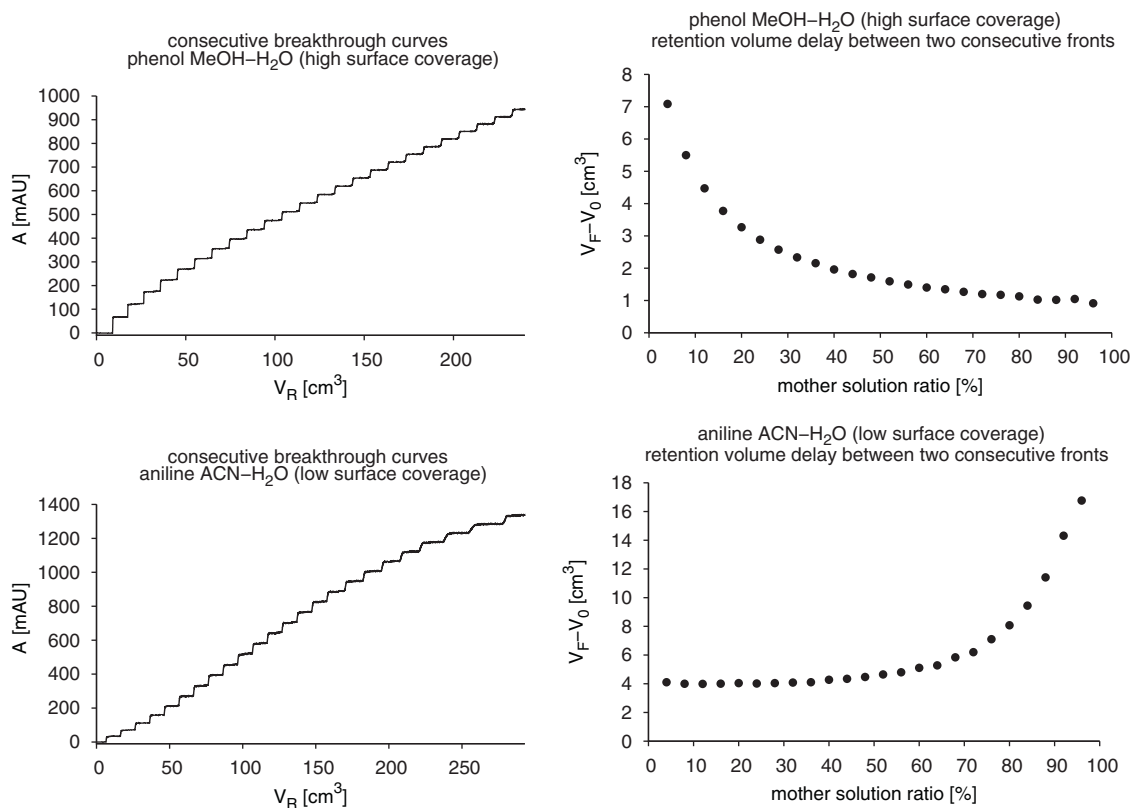
Fig. 1. Example consecutive breakthrough curves of phenol from methanol–water mobile phase on column with high surface coverage (3.27 µmol/m^2), and aniline from acetonitrile–water mobile phase on column with low surface coverage (1.11 µmol/m^2). On the left the corresponding retention volume delays of the consecutive fronts are plotted. The different shape of the volume delay plots indicates different adsorption behavior.
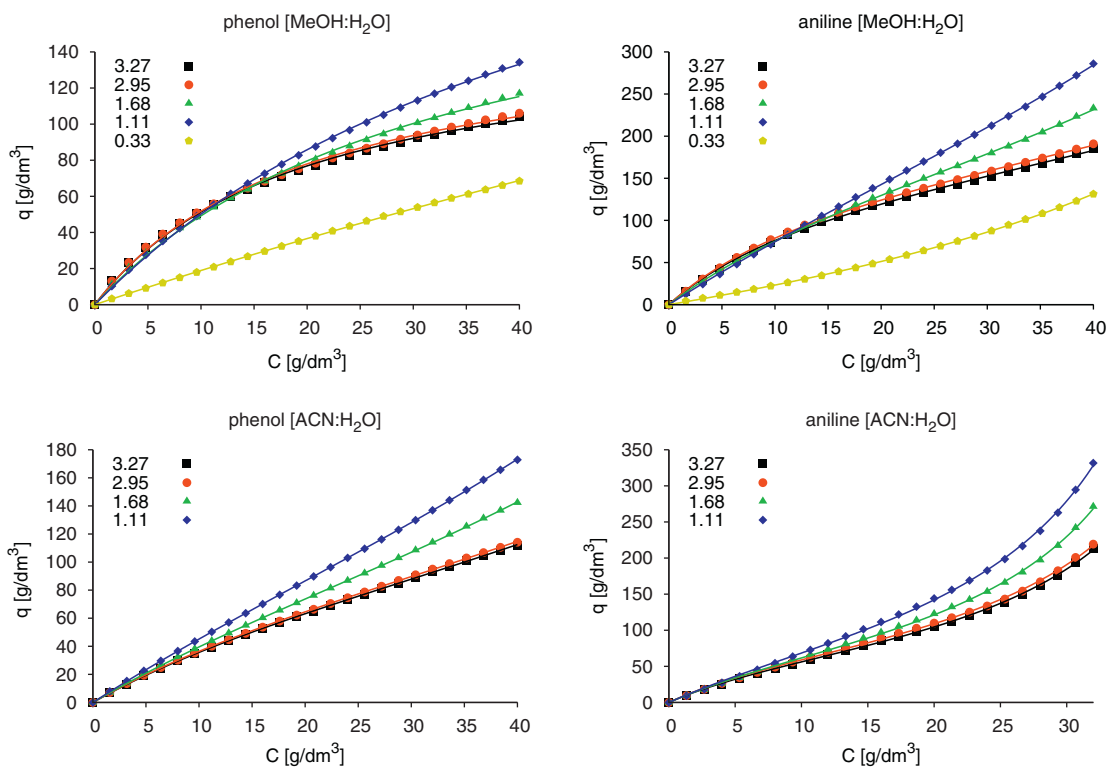

Fig. 2. The raw adsorption isotherm data points and the best fitted isotherm models for phenol and aniline from methanol–water and acetonitrile–water mixture on non-endcapped columns, with surface coverage of 3.27, 2.95, 1.68, 1.11 and 0.33 µmol/m^2.

Table 2
The best fitted isotherm parameters derived from the nonlinear fitting of the isotherm models to the raw adsorption isotherm data points of phenol and aniline from two different mobile phases on five non-endcapped reversed phase columns with different surface coverage of the octadecyl ligands.

$\alpha_{C_{18}}$ (μmol/m^2)	q_{s1} (g/dm^3)	b_{s1} (dm^3/g)	q_{s2} (g/dm^3)	b_{s2} (dm^3/g)	RSS
Phenol 40 g/dm^3 [MeOH:H$_2$O]					
3.27	64.07	0.1176	152.8	0.0126	0.0329
2.95	71.38	0.1059	165.4	0.0103	0.0311
1.68	53.24	0.0941	252.9	0.0105	0.0604
1.11	32.86	0.0855	346.9	0.0114	0.0286
0.33			564.4	0.0035	0.9042

$\alpha_{C_{18}}$ (μmol/m^2)	a	q_s (g/dm^3)	b_s (dm^3/g)	b_L (dm^3/g)	RSS
Phenol 40 g/dm^3 [ACN:H$_2$O]					
3.27	4.294	112.1	0.0383	0.0078	1.234
2.95	4.370	115.6	0.0378	0.0077	1.673
1.68	4.442	136.6	0.0326	0.0090	1.112
1.11	4.867	182.2	0.0267	0.0087	0.6416
Aniline 40 g/dm^3 [MeOH:H$_2$O]					
3.27	11.37	169.1	0.0673	0.0068	28.68
2.95	11.63	182.6	0.0637	0.0063	20.92
1.68	9.652	189.6	0.0509	0.0093	20.48
1.11	8.091	291.1	0.0278	0.0088	13.92
0.33	2.134	175.5	0.0122	0.0100	2.313
Aniline 33 g/dm^3 [ACN:H$_2$O]					
3.27	8.161	78.55	0.1039	0.0207	34.65
2.95	8.642	82.30	0.1050	0.0205	46.90
1.68	8.168	89.66	0.0911	0.0218	57.04
1.11	8.106	110.1	0.0736	0.0219	78.85

equation gives better fit. It seems that there is a threshold of the octadecyl surface coverage when the adsorption equilibrium constants of the high- and low-energy sites become indistinguishable using the frontal analysis, and the adsorbent surface becomes apparently homogeneous.

When the isotherm curvature is small, the applied analyte concentration is far from the saturation capacity, and the numerical determination of the isotherm parameters becomes ambiguous. In the case of the column with the lowest surface coverage, the isotherm parameters determined using the Levenberg–Marquardt algorithm are rough estimations.

The comparison with data of phenol adsorption on endcapped columns [18] shows an opposite trend in changing the saturation capacity of the high-energy sites (q_{s1}). With the decrease of the surface coverage of the octadecyl ligands, the saturation capacity of the adsorbent increases in the case of an endcapped column, while the q_{s1} value is decreasing in the case of non-endcapped adsorbent. This suggests that the partition processes are more favorable if the surface has only a limited concentration of residual unreacted polar groups, but has mainly hydrophobic character due to the endcapping. The saturation capacity of the low-energy site q_{s2} changes in the same manner in the case of endcapped or non-endcapped adsorbents. The gradient of the increase is much higher in the case endcapped columns, which indicates that the more hydrophobic pore volume increases the enrichment of phenol molecules in the pore volume more strongly than in the case of the more polar pores of non-endcapped columns.

4.1.1.2. ACN–H$_2$O mobile phase. The adsorption of phenol from acetonitrile–water mixture shows type II adsorption isotherm shape: convex upward at low concentrations and convex downward at high concentrations. The convex upward and downward regions are divided by the inflection point of the isotherm. The inflection point of the isotherms shifts toward lower equilibrium mobile phase concentration C of the analyte with the decrease of the surface coverage. The on-set of the interaction between the solute molecules is observed at lower concentrations as the number

of residual silanol groups increases, and as the hydrophobicity of the column bed decreases.

The type II adsorption isotherms were modeled using the BET model, since the Moreau and the bi-Moreau isotherms are not suitable to describe our experimental data. When the Moreau or the bi-Moreau isotherm is fitted, the isotherm parameters do not converge to well-defined values, thus the uncertainty of the fitted parameters is large.

The Henry constant of the adsorption, a, in the case of methanol–water mixtures decreases significantly with the decrease of the octadecyl ligand density. When the mobile phase is acetonitrile–water, a slight increase can be observed. The monolayer saturation capacity q_s of the column bed is increasing with the decrease of the surface coverage, and significantly reduces when methanol is replaced by acetonitrile. The retention of phenol is governed by several individual mechanisms, hydrophobic interactions play significant part under reversed phase conditions. When methanol is replaced by acetonitrile – which interacts more strongly with the alkyl-chains – a significant enrichment in the hydrophobic layer of ligands is found. This enrichment hampers the access of the solute molecules to hydrophobic adsorption centers.

In comparison with the data recorded on endcapped adsorbents [18] the q_s parameters change the same manner with the change of the surface coverage. On the columns with same surface coverage, the monolayer saturation capacity decreases when the column is endcapped. This behavior confirms that the acetonitrile enrichment – which is much more significant on endcapped columns – hampers the access of phenol molecules to the deep segments of the bonded phases, which decreases the monolayer saturation capacity of the column.

4.1.2. Adsorption of aniline
4.1.2.1. MeOH–H$_2$O mobile phase. When we use aniline as a test compound, its weak basic character allows us to map the effect of the residual polar groups on the adsorption processes. The most significant difference in the comparison with phenol is

that aniline shows type II adsorption isotherm shape even from methanol–water mixtures.

The adsorption equilibrium constant, b_s, describes the interaction strength between the analyte and the adsorbent surface. Aniline interacts stronger with the adsorbent from methanol–water mobile phase in comparison with phenol. The equilibrium constant, the monolayer saturation capacity, and the Henry constant only slightly differ whether phenol or aniline is the probe compound from methanol–water mixture. Since the affinity of acetonitrile to residual silanols is much weaker than that of methanol [29,27], methanol molecules shield the residual silanols with water molecules more effectively than acetonitrile does.

Because of the shielded silanol groups, the difference between the polar interactions – in the case of a weakly polar and a weakly basic test compound – is slightly pronounced. A more detailed insight is provided by the analysis of the isotherm data derived from acetonitrile–water mixture.

4.1.2.2. ACN–H_2O mobile phase. The effect of the stronger polar interactions between residual silanols and the amine group of aniline can be tracked in the case of acetonitrile–water mixture binary mobile phase. The comparison of the isotherm parameters derived using the two test compounds gives a deeper insight to the role of the residual silanol groups.

Using the same organic modifier, aniline shows larger Henry constant a, but in acetonitrile–water, the magnitude is nearly twice as large as in the case of phenol, and remains within the range of the numerical error of the parameter estimation in the whole range of the studied surface coverage density.

The difference in the b_s parameter is significant if we compare the data obtained with acetonitrile–water mixture. The adsorption strength of phenol decreases with the surface coverage if we change from methanol to acetonitrile, while in the case of aniline an opposite trend is observed. It seems that when the silanols are shielded with water-rich layer, in the case of acetonitrile–water mobile phase, the basic interactions are more favorable. On the other hand, the interaction with the phenolic group is reduced significantly, because of the stronger electrostatic repulsion.

The b_L parameter represents the interaction between the adsorbent molecules. Its value is the function of the chemical properties of the analyte, and its solubility in the dividing sphere between the stationary and mobile phase. With the decrease of the surface coverage, higher water enrichment has to be assumed on the surface of the stationary phase and the solubility of phenol and aniline slightly decreases. This results in the moderate increase in the numerical value of the b_L parameter.

4.2. Overloaded elution band shapes in the coverage density space

4.2.1. Methodology for the calculations and measurements of overloaded elution bands

With the understanding of the change of the isotherm parameters and the total porosity with the surface coverage, it is possible to model overloaded elution bands for any surface coverage. In the knowledge of the function describe the connection between the isotherm parameters and the surface coverage, the parameter-set of the isotherm describe the adsorption process on any surface coverage can be calculated. During all numerical simulations, the Martin–Synge algorithm performed one thousand calculation cycles, i.e. the column was divided up to $N = 1000$ theoretical plates. For the estimation of the boundary conditions, the column was replaced by a zero-volume connector, and the same injections were performed; then a cubic spline was fitted to the recorded

chromatogram. This spline was the input for the first calculation cycle.

4.2.2. Validation of the adsorption isotherm parameters derived from FA measurements using simulated band profiles

The inverse method gives an alternative way to validate the isotherm model and the parameters derived from the model fit to the frontal analysis data. In this section we present the comparison of the measured and simulated chromatograms of overloaded elution bands. During the calculations we used the isotherm parameters listed in Table 2. The parameters remain intact during the calculation cycles, no optimization has been made. Fig. 3 compares the measured and simulated band profiles of phenol and aniline from methanol–water and acetonitrile–water, on the highest and on the column with the lowest surface coverage the isotherm parameters were determined by frontal analysis.

4.2.2.1. Simulation of high-concentration peaks of phenol. As we have shown in a previous study [18], the overloaded elution bands of phenol recorded from methanol–water mobile phases remain tailing for the entire coverage density range, but whether the band profile changes from a tailing one to a fronting one depends on the strength of the organic modifier.

From methanol–water mobile phase with the decrease of the surface coverage the broadening of the tailing part decreases, and the sharp front becomes diffuse-like. The measured and calculated band profiles are in good agreement, which validates the selected bi-Langmuir and BET isotherm models.

Using an acetonitrile–water mixture as mobile phase, with the decrease of the surface coverage a moderate change can be observed in the peak shape. At high sample concentrations, a small fronting part appears at low coverage densities. The simulated band profiles show moderate agreement with the measured band profiles at high sample concentrations.

4.2.2.2. Simulation of high-concentration peaks of aniline. In the case of the weak base aniline, even from methanol–water mobile phase on columns with high surface coverage a fronting part can be observed at high sample concentrations, with a significant tailing part at lower concentrations. This fronting part is much more pronounced than the one observed for phenol. With the decrease of the octadecyl surface coverage, the tailing part disappears and the overloaded elution band shape changes to a fronting type. The simulated peaks are in moderate agreement with the measured peaks at high surface coverages when the sample size is big, but in good agreement when the surface coverage is low.

In the case of acetonitrile–water mixture on columns with high surface coverage, the tailing part reduces significantly and appears only at low sample concentrations. In contrast, the fronting part of the peak is more dominant and appears at lower sample concentrations compared with the peak observed from methanol–water mobile phase. At low surface coverage, this mixed shape becomes clear fronting-type. The simulated peaks are in good agreement with the measured ones.

4.2.3. The change of the critical experimental parameters with the surface coverage

The shape and position of elution bands under nonlinear conditions play important role in preparative applications of chromatography. The modeling of the overloaded elution bands is crucial in these studies. To calculate overloaded elution bands for any possible coverage density, first we need to determine the connection between the column total porosity (ϵ_T), the parameters of the desired isotherm equation and the octadecyl coverage
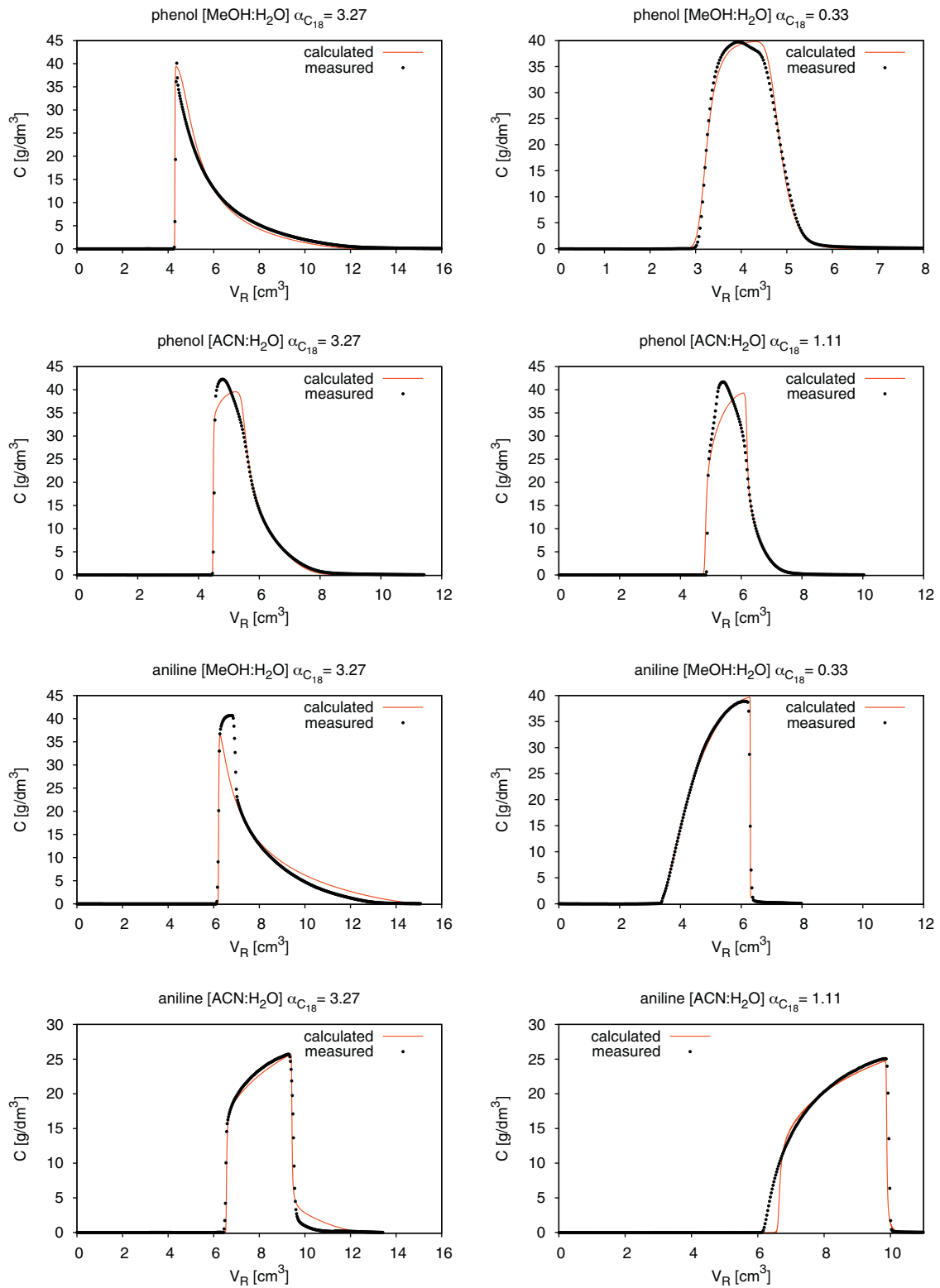
Fig. 3. Measured and computer simulated band profiles of phenol and aniline on high and low surface coverage with uniform 1.5 cm^3 injected volume.

density. Fig. 4 illustrates the linear dependence of the total porosity on the surface coverage. In the knowledge of the slope and the intercept, the porosity of a column can easily be determined for any desired coverage density. The change of the isotherm parameters against the coverage density is illustrated in Fig. 5. In the case of methanol–water mixture parameter a, in the case of

acetonitrile–water mixture parameter q_s was modeled using the following nonlinear function:

$$f(\alpha_{C_{18}}) = (p_1 \alpha_{C_{18}} + p_2) + \frac{p_3 \alpha_{C_{18}}}{(1 + p_4 \alpha_{C_{18}})} \tag{7}$$
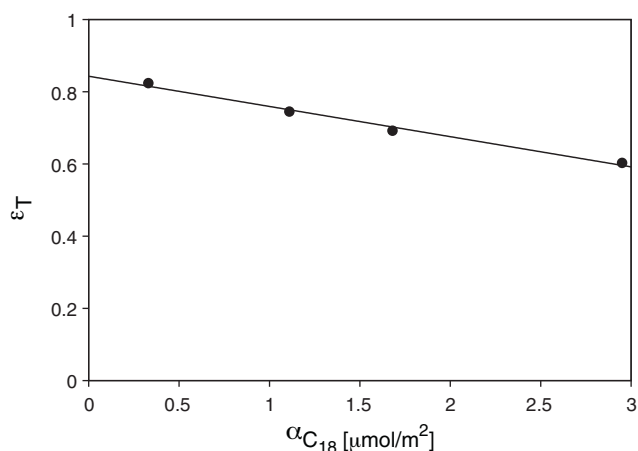
Fig. 4. The change of the total porosity (ϵ_T) of the non-endcapped columns with the change of the surface coverage of the octadecyl ligands ($\alpha_{C_{18}}$).

where p_1, p_2, p_3 and p_4 are the fitted parameters. In the case of parameters b_s and b_L linear regression was used to model their dependency on coverage density.

4.2.4. Simulation of high-concentration elution bands in the coverage density space

We demonstrate the change of the band shapes with the decreasing polarity of the column bed in the case of aniline as test molecule in the case of methanol–water and acetonitrile–water mixtures.

The elution bands of aniline recorded from methanol–water mobile phases (see Fig. 6) do not follow the uniform tailing trend observed in the case of phenol from the same eluent composition. The deviation appears as a fronting part in the chromatogram,
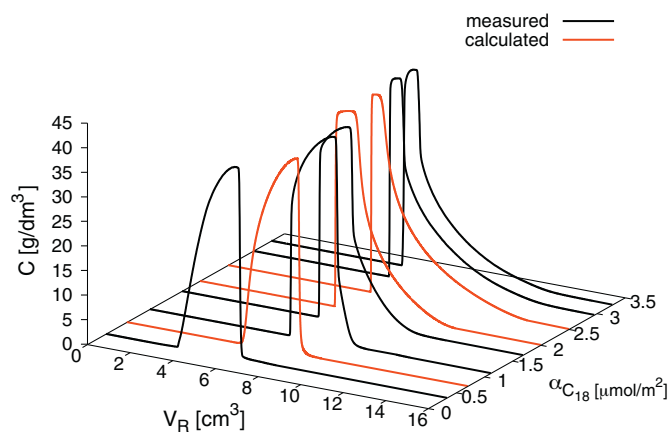
Fig. 6. Measured and simulated overloaded elution bands of aniline from methanol–water mixture in the C_{18} coverage density space. Chromatograms were measured on five columns with different surface coverage – see Table 1. The simulated peaks were calculated using the estimated isotherm parameters in the coverage density phase, using the parameter set on a column with theoretical surface coverage, and the mass balance equation was solved by means of the Martin–Synge algorithm The injection volume was uniformly 2.0 cm^3, the injected concentration was 40 g/dm^3.

which indicates the multilayer adsorption behavior of aniline. The solute–solute interactions become stronger with the decrease of the surface coverage of the octadecyl chains, and with the parallel increase of the number of the residual polar groups. With the increase of the polarity of the column bed, the sample concentration limit where the tailing part appears decreases, and below a surface coverage threshold the tailing part disappears. Parallel with the decrease of the tailing part, the fronting type strengthens and at lower surface coverages a fronting peak can be observed. This phenomenon is connected with the number of accessible unreacted
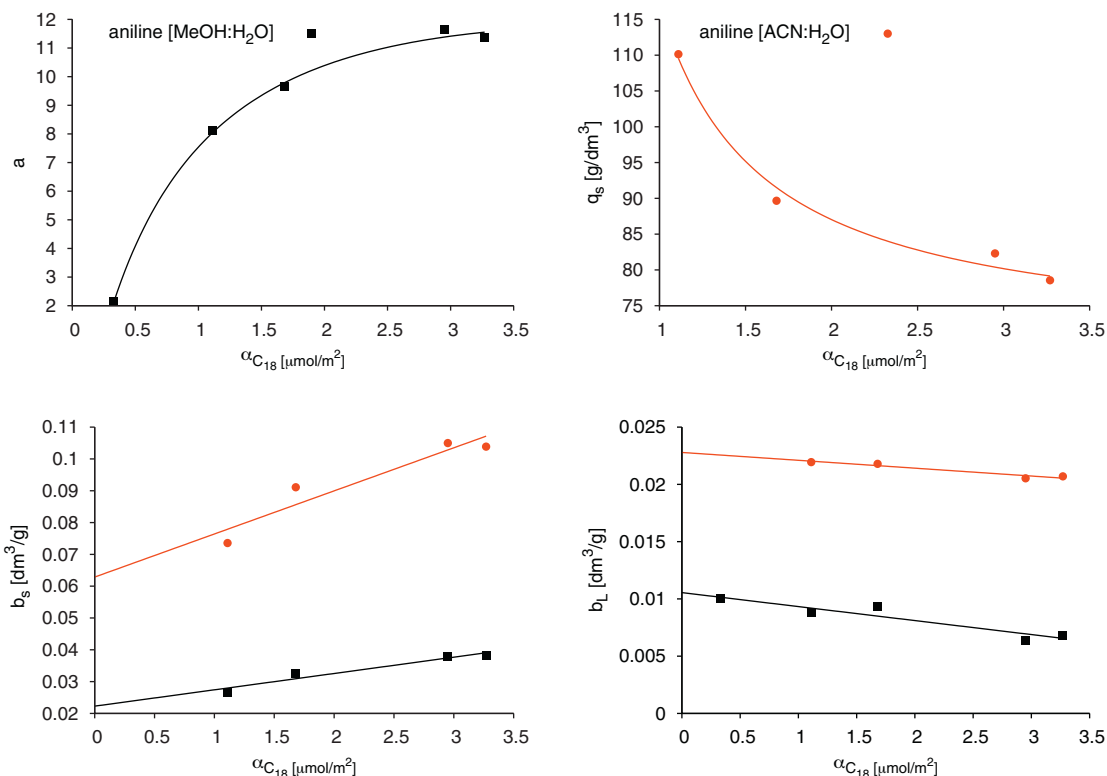

Fig. 5. The best fitted adsorption isotherm parameters, and the best fitted functions describe the relation between the isotherm parameters and surface coverage. The circle symbol indicates data from acetonitrile–water, and the square indicates data from methanol–water mobile phases.
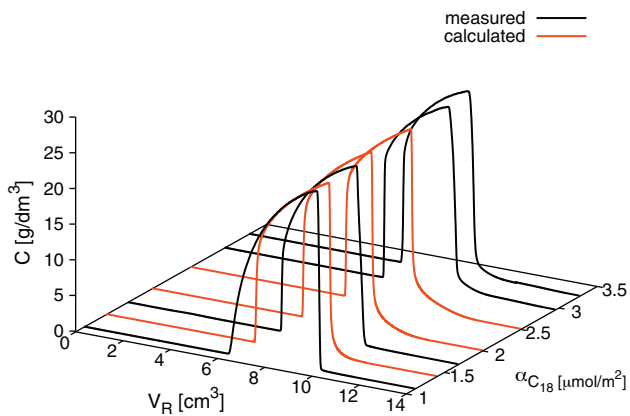
Fig. 7. Measured and simulated overloaded band profiles of aniline recorded from acetonitrile–water mobile phase. Injection volume was $2.0\,\mathrm{cm^3}$, the injected concentration was $33\,\mathrm{g/dm^3}$.

Fig. 9. The change of the retention factor (k') of aniline and phenol with the coverage density from different mobile phases.

residual silanols, and the interaction between them and the test molecule. The mixed type retention is what cause the disagreement between the ideal simulated and measured band profiles in Fig. 3.

When one applies acetonitrile as organic modifier, the effect of the silanol groups can be observed more explicitly. The non-protic properties of acetonitrile result in weaker apolar shielding than in the case of methanol. This is confirmed by the chromatograms of aniline recorded from acetonitrile–water mixture and plotted in Fig. 7. In this case only a small tailing can be observed, only on columns with higher surface coverage, what disappears on columns with medium surface coverage. Basically the bands have fronting shape even at higher surface coverage. Whether or not multilayer adsorption takes place, depends on the injected concentration in the case of type II adsorption isotherms. This concentration threshold appears on the isotherms as an inflection point.

4.2.5. Effect of column loading on the overloaded elution band shapes

The column overloading was described using the loading factor (L_f) [20]:

$$L_f = \frac{C_{\mathrm{inj}} \times V_{\mathrm{inj}}}{(1 - \epsilon_T) \times V_G \times q_s} \tag{8}$$

where C_{inj} and V_{inj} are the injected concentration and volume, respectively.

Fig. 8 shows a series of overloaded elution bands of aniline measured from methanol–water mixture on the column with surface
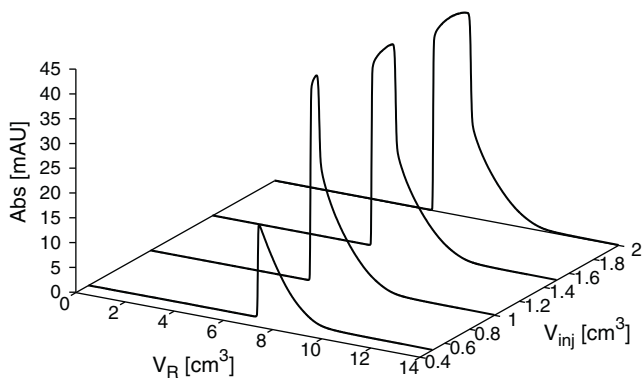
coverage of $1.68\,\mathrm{\mu mol/m^2}$ with different loading factors. When the loading factor is below a threshold, tailing peak shape can be observed. Above the threshold, the front of the band starts to deviate from the ideal tailing one, and with the further increase of the injected sample size, the fronting appears at high concentrations with the presence of a tailing at lower concentrations. This agrees well with the BET-type adsorption behavior.

4.3. Retention pattern of phenol and aniline

Fig. 9 shows the dependence of the retention factor (k') of phenol and aniline on surface coverage from the two applied mobile phases. The retention factor under linear conditions can be calculated by means of the initial slope of the isotherm and the phase ratio (F) of the column:

$$k' = Fa \tag{9}$$

The increasing tendency of k' values represents the effect of the strengthening hydrophobicity and weaker possibility for polar–polar interactions with the higher surface coverage of the octadecyl ligands. The change of the organic modifier from methanol to acetonitrile reduces the retention factor of both phenol and aniline. The difference between the retention factor data is the highest on the 3.27-$\mathrm{\mu mol/m^2}$ column, and become almost identical in the case of aniline, and close to each other in the case of phenol on the 1.11-$\mathrm{\mu mol/m^2}$ column. The use of acetonitrile as organic modifier – due its weaker interaction with residual silanols – makes the polar groups more accessible for analyte molecules, and the reduced possibility of hydrophobic interactions can be compensated more efficiently in the comparison with methanol as organic modifier. Because of this phenomenon, the gradient of the decrease of the retention factor with the decrease of the surface coverage of the C_{18}-ligands is more moderate in the case of acetonitrile–water solvent mixture. The observed identical retention factor of aniline from both solvents on the 1.11-$\mathrm{\mu mol/m^2}$ column suggests, that the basic interaction between amine group and residual silanols is more pronounced than in the case of hydroxyl group is presented.

5. Conclusions

The experimental conditions under which the adsorption isotherms were measured correspond to those preferred in HPLC analyses. Two commonly employed organic modifiers were chosen in this study to investigate their influence on adsorption and retention. In the experiments, a series of non-endcapped octadecyl

Fig. 8. Effect of the sample loading (L_f) on the overloaded band shapes of aniline from methanol–water mixtures determined experimentally on column with medium coverage density of $1.68\,\mathrm{\mu mol/m^2}$.

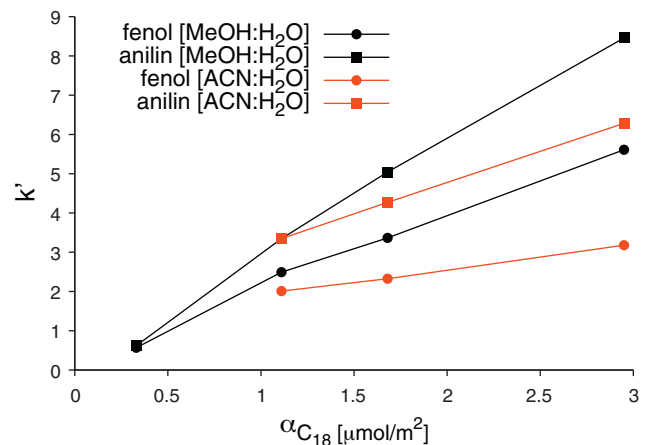bonded phases was used to investigate the role of the residual silanols in solute adsorption and retention mechanism.

The adsorption behavior of phenol is changing from Langmuir type (from methanol–water) solution to BET type (from acetonitrile–water solution). Acetonitrile is the stronger eluent and it significantly reduces the column saturation capacities as well as the retention factor of the analytes at high surface coverage. At this range of the surface coverage, the hydrophobic interactions are predominant in the comparison while the polar interactions do not have such significant effect.

The use of acetonitrile as organic modifier increases the influence of the residual silanols on solute adsorption. This effect is much pronounced in the case of the basic compound, but not negligible for weakly acidic compounds, even at low surface coverage. For the retention of aniline, the effect of acetonitrile is rather significant. The residual silanols are uncovered and accessible for interaction with aniline, whereas when methanol is used as organic modifier, the residual silanols may be covered by a mixture of methanol and water. Using acetonitrile solution as a mobile phase, the silanol groups are covered by a much more water-rich layer. This more polar layer cannot shield effectively the silanol groups and they will be accessible much more for polar interaction. This phenomenon modifies the shape and the position of the overloaded elution bands.

Acknowledgments

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