UNIVERSITY OF PÉCS

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Investigation of retention mechanism of cavitands in reversed phase chromatography and the study of the adsorption of water in hydrophilic interaction liquid chromatography

PhD thesis

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To Emőke and Nimród,

for your love and support.

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1 Introduction

"..., it is more important to know how it works than how to do it; basics rather than recipes."
J. Calvin Giddings

Chromatography is present in all areas of everyday life. Over the last century, a lot of chromatographic methods have been developed, ranging from food industry to toxicology or to space research. For example, the use of chromatographic techniques is indispensable in the pharmaceutical production process. In addition, besides the quantitative and qualitative analyses, the method is applied for tracking reaction pathways and for detecting degradation processes. Chromatographic methods have been developed for the detection of chemical residues in the food industry and for the determination of different active substances. In the field of toxicology, I would mention the drug testing and analysis. New designer drugs emerge every day. The various chromatographic techniques have a large role in determining the structure of drug molecules. In addition, the doping agents those athletes use can be detected and qualitatively and quantitatively analyzed using various chromatographic techniques. From the environmental analytical point of view, chromatographic methods also play a huge role in the determination and detection of polycyclic aromatic hydrocarbons (PAHs), pesticide residues and other toxic and nontoxic compounds. Chromatographic studies in research laboratories, such as studying the interactions between stationary phases and samples, make a significant contribution to solving everyday problems.

Methods of purification and separation like chromatography have already been applied in ancient times, but most researchers associate the birth of chromatography with the name of Mikhail Semyonovich Tswett. In 1903, he used liquid-adsorption column chromatography with calcium carbonate as adsorbent and petroleum ether/ethanol mixtures as eluent to separate chlorophylls, xanthophylls, carotene fractions. These fractions formed colored bands on the adsorbent. The name "chromatogram" was the name of the adsorbent containing color rings (from Greek χρώμα=chroma and

γραφειν = grafein, color and writing). His discovery was published in 1906 by a journal of the German Botanical Society [1]. The color of the sample has no significance for the chromatographic techniques however the name of “chromatography’’ has become accepted.

László Zechmeister (at the University of Pécs) used the adsorption chromatography in his carotene studies in the 1920s and 1930s. The first chromatography textbook in the world was written by László Cholnoky and László Zechmeister in 1937. The work was published in Vienna [2]. The “written history” of the chromatography is a hundred years old although the current chromatographic methods are based on the results of the chromatographic developments of the last fifty years. Hungarian scientists also participated in the developments; many of them became the dominant figures of the chromatography. The most prominent among them are István Halász, Csaba Horváth, Ervin Kováts, László Ettre and László Szepesy. According to rumors, in professional circles they were called "Hungarian mafia". All of them were active in different fields of chromatography. Some of them focused on theoretical work, while others concentrated practical achievements.

In the late 1960s, there was a need to increase the speed and efficiency of liquid chromatography methods. It was achieved by using small-particle columns those required high-pressure pumps. This ultimately led to the construction of the first High Performance Liquid Chromatography (HPLC) device by Csaba Horváth and Lipsky. In 1966 they had already used the current denomination of the method in the first article [3]. The development of HPLC has contributed greatly to the work of Snyder [4], Kirkland [5] and Huber[6].

HPLC became popular because it can be used analytical and preparative modes. Furthermore it also can be used to detect non-volatile compounds (about 80 percent of all known compounds) having a molecular weight ranging from a few hundred Dalton to 10^9 Dalton and its versatility and precision rendered it virtually indispensable in pharmaceuticals as well as other diverse industries. In analytical or linear chromatography, as the goal is qualitative and quantitative analysis, the compounds (and the rest of the sample) are simply diverted to waste or to a ‘destructive’ detector such as a mass spectrometer or

charged aerosol detector. However in preparative or non-linear chromatography is used for the characterisation of separation materials and for optimization of large scale separation processes, as well as in the isolation and purification of high value compounds. With high concentrations it is also possible to determine adsorption isotherms which are useful tools for quite a wide variety of reasons. It can be used for characterization of chromatographic separation systems and then gives information on the retention mechanism; moreover, they can be used for the study of unexpected chromatographic phenomena. If the adsorption isotherm is known it is also possible to calculate chromatograms and subsequently optimize the separation process numerically. The advantage of a numerically optimized separation process saves time, money and the environment.

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2 Aims and overview

The understanding of the retention behavior of medium or large molecules is an area of interest in liquid chromatography. The investigation of the chromatographic behavior of resorcinarenes and cavitands is meager up to now. To understand the chromatographic behavior of cyclic oligomers (such as resorcinarenes and cavitands) the temperature [7-9] and pressure [10] dependence of retention factors of these analytes was investigated. Furthuremore the thermodynamics of the retention in acetonitrile-water and methanol-water mobile phase for cyclic oligomers were compared. Moreover their retention mechanism was investigated by generating of adsorption isotherm, applying nonlinear chromatographic methods [8].

Besides the investigation of retention mechanism of resorcinarenes and cavitands on reverse stationary phases, the adsorption of water in hydrophilic interaction chromatographic system has studied, on Cogent Silica C and Cogent Phenyl hydride stationary phases at different temperatures [11].

The main objectives of this PhD research were:

- determination the thermodynamics (changes of enthalpy, entropy and the Gibbs-free energy) of the retention of resorcinarenes (HRM, MRM) and cavitands (HCM, MCM) in different reverse phased chromatographic system
- determination the equilibrium adsorption isotherms paramerets of resorcinarene (HRM) and cavitand (MCM) by inverse method and compare the Henry constants obtained from adsorption isotherm determination and thermodynamics determination
- exploration the influence of the change of molar volume on the retention of resorcinarene (HRM) and cavitand (MCM) on alkylsilyl and polar-embedded C_8 and C_{18} reversed phases

- investigation the adsorption of water in aqueous normal-phase liquid chromatography on Cogent Silica C and Cogent Phenyl hydride stationary phases at different temperature

3 Literature review

3.1 Resorcinarenes and cavitands in chromatography

Resorcinarenes are cyclic oligomers those are synthesized by the condensation of resorcinol and various aldehydes. During the late 19th century Baeyer reported that the condensation reaction of resorcinol and benzaldehyde gave a mixture of products which were beyond the characterization methods of that time. His effort was to develop new dyes [12, 13]. Högberg and his colleagues reinvestigated the reaction [14] and further developments were made by Cram and his colleagues [15]. The pioneering researches of Cram [16], Pedersen [17] and Lehn [18] in host-guest chemistry led to the discovery of one molecule inside another and the concept of container molecules. Yasuhiro and coworkers noted firstly that resorcinarenes could be host molecules in the host-guest interaction [19].

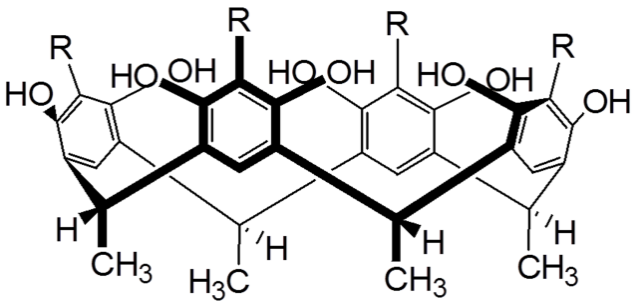
The official IUPAC name for resorcinarene containing alkyl groups on the lower rim is 2, 8 ,14 ,20-tetraalkylpentacyclo[19.3.1.1.11.]octacosa-1(25),3,5,7(28), 9,11,13(27)15,17,19(26),21,23-dodecene-4,6,10,12,16,18,22, 24-octol [17]. The name “resorcinarene” was suggested by Schneider [20]. Gutsche [21] and Böhmer [22] referred to them as calix[4]resorcinarenes classifying them as calixarenes. As the official IUPAC name of alkylene-bridged Resorcinarenes are very complicated, they are commonly called cavitands [15].

By bridging the neighboring hydroxyl groups of the resorcinarene, one can make a new type of macrocycle with an extremely rigid cavity. The name “cavitands” was given by the Nobel-laureate Donald J. Cram to those classes of synthetic compounds, which contain an enforced concave cavity which is large enough to accommodate simple molecules or ions. Therefore, the resorcinarenes and their derivatives can also be considered cavitands like cyclodextrins and calixarenes, because of their cavity-shaped structure. It is an interesting coincidence, that alkylene-bridged resorcinarenes are also referred to as cavitands [23].

I want to mention here that one part of my study directed to two types of the above-mentioned macrocyclic, which have hydrogen atoms or methyl groups (R = H or CH_3) on the upper rim (Figure 1).

I denote the C-tetra-methylcalix[4]resorcinarene HRM, the 2-methyl-C-tetra-methylcalix[4]resorcinarene MRM, the cavitand HCM and the methyl-cavitand MCM. Each compound has methyl groups on the lower rim. There are some structural differences between the two types of cyclic tetramers. The conformationally mobile resorcinarenes can adopt five different conformers, while the structures of the cavitands are conformationally more rigid, because of the methylene bridges between the oxygen atoms of the hydroxyl groups. On the other hand, the resorcinarenes can take part in hydrogen bonding with polar functional groups by their hydroxyl groups. The methylene-bridged cavitands are apolar structures.

A



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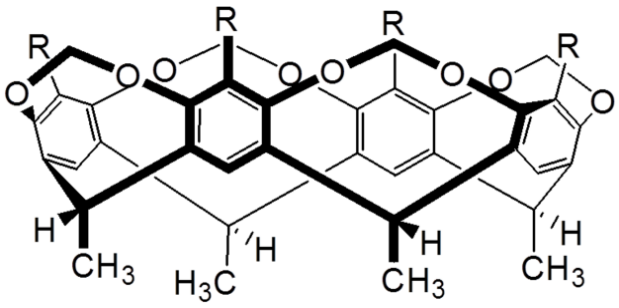

Figure 1. Structures of resorcinarenes and cavitands.

The ligands are (A) R = H in HRM R = CH_3 in MRM and (B) R = H in HCM and R = CH_3 in MCM.

The resorcinarenes and cavitands are relatively easy to synthesize in a wide range of size and with different functional groups [24-26]. The ability to create host-guest interactions and many possible structural variations allows them to have applications in supramolecular recognition [27, 28], nanoscale reaction containment [29], catalysis [30], chiral NMR shift agents [31-33], chromatographic separations [34-38], drug encapsulation, drug protection and delivery [39] and mediating endocytosis [40].

In spite of the numerous applications of cavitands in a broader sense, their analysis and separation with HPLC, particularly the study of their chromatographic behavior in reversed phase liquid chromatography, is not widespread.

Up to now, the role of HPLC was to control the process of the synthesis. Ming and his colleagues reported on the thermolytic reaction of C-tetra-methylcalix[4]resorcinarenes followed by reversed phase HPLC analysis using gradient elution with water/MeOH (50:50 v/v%) to 100% MeOH. The synthesis provided the formation of numerous new products [41]. The tetra(alkoxymethyl) derivatives of resorcinarene synthesized via the Mannich reaction were analyzed with HPLC on a C_{18} column [42]. Warmuth and Liu synthesized nanocontainers, which were built up from six bowl-shaped cavitands and purified with reversed phase HPLC using protein and peptide C_{18} columns[43]. This team carried out semi-preparative HPLC purification of hemicarceplexes formed by hemicarcerand host and guest molecules with dichloromethane/ether (100:1 v/v%) as the mobile phase and silica stationary phase [44].

3.2 Determination of retention mechanism in linear chromatography

The interactions between the analytes and chromatographic phases depend on the structure of the analyte and the quality of the mobile and stationary phases inclusive of the functional groups of the solute and stationary phase. These interactions are responsible for the retention behavior of the analytes. Temperature has also a strong effect on the retention mechanism. Thermodynamic properties for the transfer of the analyte from the mobile phase

to the stationary phase can elucidate on the retention mechanism. In the investigation of chromatographic retention, the elucidation of thermodynamic information often takes the form of van't Hoff analysis. One of the first applications of the van't Hoff law in the field of chromatography was the chiral separation of enantiomers by gas chromatography [45, 46]. Many previous studies have been directed to the temperature effect on retention and their territory is expanded to a variety of systems. While the publications of Jensen and his colleagues [47] and Dorsey and coworkers [48] give a general scope to the van't Hoff equation and the molecular mechanism of retention, Ranatunga and Carr examined the retention thermodynamics of small non-polar molecules and determined the enthalpic changes in the stationary phase based on the formation of lipophilic interaction between solutes and the stationary phase ligands [49]. The study of the thermodynamic properties of solute transfer was carried out by Lee and Cheong on a carefully designed chromatographic system including a squalene impregnated C_{18} stationary phase [50].

In the 1960s Giddings suggested that changes in molecular volume of analyte could produce changes in the equilibrium distribution of the analyte between the mobile and stationary phases [51]. The magnitude of the shifts depended on the analyte – solvent interconnection and on the changes of the structure of the chromatographic phases. The chromatographic parameters that are supposed to be constant, such as retention factor, column porosity, hold-up volume, mobile phase density and viscosity all depend on pressure, as it was described before in the theoretical analysis of Martin and Guiochon [52]. Early studies reported that the increase of retention factor with pressure for small neutral molecules was generally small in reversed-phase HPLC [53-55]. Larger increase was observed for ionizable compounds at the average column pressure of 500 bar [56]. It has been demonstrated that charged and ionizable components expose much stronger pressure influences on retention. These effects have been recently systematically modelled for and isolated from the type of neutral compound effects we have in this study [57]. Åsberg et al. suggested a practical approach was to predict the retention shifts due to pressure and temperature gradients in ultra-high-pressure liquid chromatography [58]. Tanaka and his colleagues [59] observed a decrease in

retention with increasing pressure for some analytes at mobile phase pH close to the solute pK_a. Recently, numerous works demonstrated the strong influence of pressure on retention for large molecules such as proteins [60-62]. The effect of pressure on retention of cavity-shaped molecules has been scarcely studied so far. Only a few publications [54, 63] can be found on this topic, where β-cyclodextrins as mobile phase additives were applied to study the pressure-induced retention increase of nitrophenols and the changes in retention with pressure were studied on β-cyclodextrin stationary phases [64, 65].

3.2.1 Determination of the thermodynamic parameters in linear chromatography

The retention mechanism of the adsorption or partition process of the analyte on the stationary phase consists of assuming thermodynamic equilibrium of analyte between the stationary and the mobile phases. At chemical equilibrium or in phase equilibrium the total sum of chemical potentials, μ_i (also called partial molar free enthalpy) is zero, as the free energy is at a minimum. If both temperature and pressure are held constant, we can write:

$$dG = dG^S + dG^M = \sum_{i=1}^{N} \left(-\mu_i^S + \mu_i^M \right) dn_i = 0 \qquad \text{Eq. 1}$$

Well-known that, the chemical potential of a substance *i* is expressed in term of its concentration. The relationship between them is as follows:

$$\mu_i = \mu_i^0 + RT \ln c_i \qquad \text{Eq. 2}$$

$\mu_i°$ is the chemical potential of *i* in its standard state. By the washing of the stationary phase with the mobile phase (which means ‘mechanical stirring’ of both phases), the system is equilibrated. The distribution equilibrium of analyte between the two mentioned phases is given by writing the equality of its chemical potentials in two phases. The state of equilibrium partitioning is attained when the solute concentrations in both phases no longer change with time.

$$\mu_i^S = \mu_i^M \qquad \text{Eq. 3}$$

$$\mu_i^{0,S} + RT \ln c_i^S = \mu_i^{0,M} + RT \ln c_i^M \quad \text{Eq. 4}$$

$$\ln c_i^S = \frac{\mu_i^{0,M} - \mu_i^{0,S} + RT \ln c_i^M}{RT} = \frac{-\Delta\mu_i^0}{RT} + \ln c_i^M \quad \text{Eq. 5}$$

$$c_i^S = \exp\left(\frac{-\Delta\mu_i^0}{RT} + \ln c_i^M\right) \quad \text{Eq. 6}$$

$$c_i^M = \exp\left(\frac{\Delta\mu_i^0}{RT} + \ln c_i^S\right) \quad \text{Eq. 7}$$

$$\frac{c_i^S}{c_i^M} = \exp\left(\frac{-\Delta\mu_i^0}{RT} + \ln c_i^M - \frac{\Delta\mu_i^0}{RT} - \ln c_i^S\right) = \exp\left[\frac{-2\Delta\mu_i^0}{RT} + \ln c_i^M - \left(\frac{-\Delta\mu_i^0}{RT} + \ln c_i^M\right)\right] \quad \text{Eq. 8}$$

$$\frac{c_i^S}{c_i^M} = \exp\left(\frac{-\Delta\mu_i^0}{RT}\right) = K \quad , \quad \text{Eq. 9}$$

where the superscripts *S* and *M* denote the stationary and the mobile phases, respectively, c_i is concentration of *i* analyte. Note that the chemical potential of the analyte *i* in its standard state is the same in both the stationary and the mobile phases.

The retention factor is the ratio of the amounts of solute in the stationary and the mobile phases:

$$k = \frac{V_S}{V_M} \exp\left(\frac{-\Delta\mu_i^0}{RT}\right) = \frac{V_S}{V_M} K \,, \quad \text{Eq. 10}$$

where *k* is the retention factor and V_S and V_M are the volume of stationary and mobile phase, respectively, *K* is the Henry's constant or simply the ratio of the analyte concentrations in the stationary and the mobile phases at equilibrium.

The thermodynamic parameters of the adsorption or partition process of the analyte on the stationary phase correlate with the retention factor under isocratic conditions. The enthalpy and entropy changes are estimated by analyzing the temperature dependence of the retention factor. The retention factor was related to thermodynamic parameters as

$$\ln k = \frac{-\Delta\mu_i^0}{RT} + \ln\frac{V_S}{V_M} = \frac{-\Delta H_i^0 + T\Delta S_i^0}{RT} + \ln\frac{V_S}{V_M}, \quad \text{Eq. 11}$$

$$\ln k = \frac{-\Delta G}{RT} + \ln\frac{V_S}{V_M} = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln\frac{V_S}{V_M}, \quad \text{Eq. 12}$$

where ΔH, ΔS *and* ΔG represent the enthalpy, entropy and Gibbs free energy change of the reversible binding of the analyte, respectively, V_S/V_M is the phase ratio. It is well known that the phase ratio strongly depends on the structure of the stationary phase – the nature of the bonded ligand strongly influences the volume of the bonded phase, thus the phase ratio – but it is often assumed to be constant at different temperatures and pressures [66].

The enthalpy change can be derived from the slope of the van't Hoff plot (Eq. 12). The more negative the value of ΔH, the more favorable is the transfer of the analyte from the mobile phase to the stationary phase, thus the stronger is the interaction between the analyte and the stationary phase. This leads to larger values of retention factor. The entropy change can be calculated from the intercept of the van't Hoff plot (Eq. 12). The less negative value of ΔS, the more favorable is the interaction between the analyte and the stationary phase. The Gibbs free energy change evaluating from the Gibbs-Helmholtz relation governs the retention.

If the retention mechanism is constant with temperature, it may be possible to compare the enthalpy, entropy and Gibbs free energy changes of a single analyte on different columns or different analytes on the same column.

3.2.2 Effect of stationary phase on the pressure induced retention changes

The dependence of the retention factor on pressure can be expressed by the equation:

$$\ln k = -\frac{\Delta G}{RT} + \ln\frac{V_S}{V_M} = -\frac{\Delta E}{RT} - p\frac{\Delta V_m}{RT} + \frac{\Delta S}{R} + \ln\frac{V_S}{V_M}, \quad \text{Eq. 13}$$

where ΔE is the internal energy of the reversible binding of the analyte. ΔV_m is the change of molar volume of the solute. The change of molar volume of the

analyte is associated with its passage from the mobile to the stationary phase. Eq. 13 suggests that a plot of ln *k* versus pressure should be a straight line with a slope of $-\Delta V_m/RT$. If the molar volume change is negative, i.e. the solute has a smaller volume in the stationary phase than in the mobile phase the retention of the analyte should increase with pressure according to the Le Chatelier's principle.

Note that the pressure increase can also change – besides the molar volume of the analyte – the structure of the stationary phase, its interaction with mobile phase and solute. However the primary factor is the change of the solute's molar volume. I consider the bulk compressibility of mobile phase minimal when moderate pressures are applied (up to 400 bar). The high pressure is concomitant with the frictional heating effects within the column. It is possible to study the effect of pressure alone by attaching narrow restriction capillaries of different length to the exit end of the column. The frictional heat can be minimized by applying constant, low flow rates (0.1 – 0.4 mL/min) and short columns packed with relatively large stationary phase particles [56].

In reversed-phased liquid chromatography, the influence of pressure on retention assuming constant flow rate arises from many sources, which include the expansion of the column tube, the compressibility of the mobile and the stationary phases, the change of the partial molar volume of the analyte and structural alteration of the alkyl-bonded layer of the silica [52, 67, 68].

The changes of the stainless-steel column tube can be considered as negligible in practice, increasing the volume of the tube by less than 0.3% over the pressure range 1 – 400 bar. The compressibility of the mobile phase may increase the hold-up volume by 1 to 5% depending on the nature of the organic modifier and its volume ratio.

The decrease in the volume of the packing material causes 0.5 – 5% increase in the retention of thiourea if the silica contains alkyl (octadecyl) chains on its surface. The neat, underivatized silica is nearly incompressible and does not show any pressure induced effect. As the change of volume of the stationary phase originates from the alteration (e.g. conformation, solvation by organic modifier) of the alkyl-bonded layer, it seems obvious that Fallas et al. have not

found any difference for the influence of pressure between the inorganic-organic hybrid and normal inorganic stationary phases [56, 69]. Similarly, a comparison of fully porous and superficially porous silica [70] concluded that both types of stationary phases yielded similar degrees of retention change when increasing the pressure.

All the effects that can influence the alkyl-bonded region may cause a volume change of the stationary phase with pressure. Gritti et al. [67, 68] compared the compressibility of the pure silica (0% carbon), non-endcapped (10% carbon) and endcapped C_{18} (20% carbon) phases. They concluded that the larger the carbon content – and in this context the surface coverage – is the influence of pressure on the volume change of the bonded silica.

Fallas et al. [71] confirmed Tanaka's observation that the polymeric C_{18} phase based on silica generated larger increase in retention with pressure than the monomeric bonded silica phase. These authors found that the pressure effects are considerably greater on C_{18} than on C_1 phase. Melvin studied the effect of the ligand type on the pressure-induced retention changes and pointed out that the more hydrophobic the stationary phase the greater is the increase of retention factors for non-polar analytes, comparing C_1, C_8, C_{18} and PFP phases [70]. Fallas et al. [71] noted that on a stationary phase containing embedded polar groups, a substantial increase in retention with pressure was obtained for hydrophilic solutes regardless of the composition of the mobile phase.

3.3 Determination of the retention mechanism in nonlinear chromatography

The classical van't Hoff plot (Eq. 12) provides information about the retention mechanism by thermodynamic properties for the transfer of the analyte from the mobile phase to the stationary phase. The retention mechanism is very complex. It originates from the complex structure of the alkyl bonded stationary phase and analytes, furthermore from the nature of interface of RPLC systems.

Linear or analytical chromatography does not give detailed information on the retention mechanism because it merely measures the retention time, which is simply the combination of the various contributions of the different interactions

between the analytes and the chromatographic phases. With nonlinear chromatography, the adsorption isotherm of the analyte can be determined by the overloading experiments. From the isotherm data, the amount of the various types of adsorption sites present on the surface can be assessed and the individual equilibrium constants can be also calculated.

As a very rough approximation, the retention process can be described on single equilibria of the analyte distribution between the mobile and stationary phases. The Henry constant is related to the retention factor as one can see at Eq. 10. In nonlinear chromatography, one can distinguish the contribution of the saturation capacity (q_S) and the adsorption–desorption equilibrium constant (*b)* to the retention factor. The saturation capacity is the amount of an analyte forming a monolayer on the surface. In the case of homogeneous surface, we can write the retention factor as:

$$k = K\frac{V_S}{V_M} = q_s b\frac{V_S}{V_M} \qquad \text{Eq. 14}$$

and more generally, for a heterogeneous surface (N-Langmuir adsorption isotherms):

$$k = \frac{V_S}{V_M}\sum_{i=1}^{N}\left(q_{s,i}b_i\right) \qquad \text{Eq. 15}$$

where $q_{S,i}$ is the abundance of adsorption site *i* and b_i is the adsorption – desorption equilibrium constant on adsorption site *i*.

3.3.1 Adsorption isotherm models

The adsorption isotherm is the equilibrium relationship between the solute analytes in the mobile phase and the analytes on the stationary phase at a constant temperature. This relationship is important to describe the interactions between the components in the mixture to be separated. The isotherms are classified five groups by Brunauer et al [72] as it seen Figure 2. The most of the adsorption isotherms measured in RPLC were found to be convex upward and to belong to type I (langmuirian isotherm) of the gas – solid equilibrium isotherm

classification of Brunauer et al. (Figure 2). Very few cases had shown adsorption data consistent with isotherms of type II to type V or to type III (antilangmuirian isotherms that are convex downward).

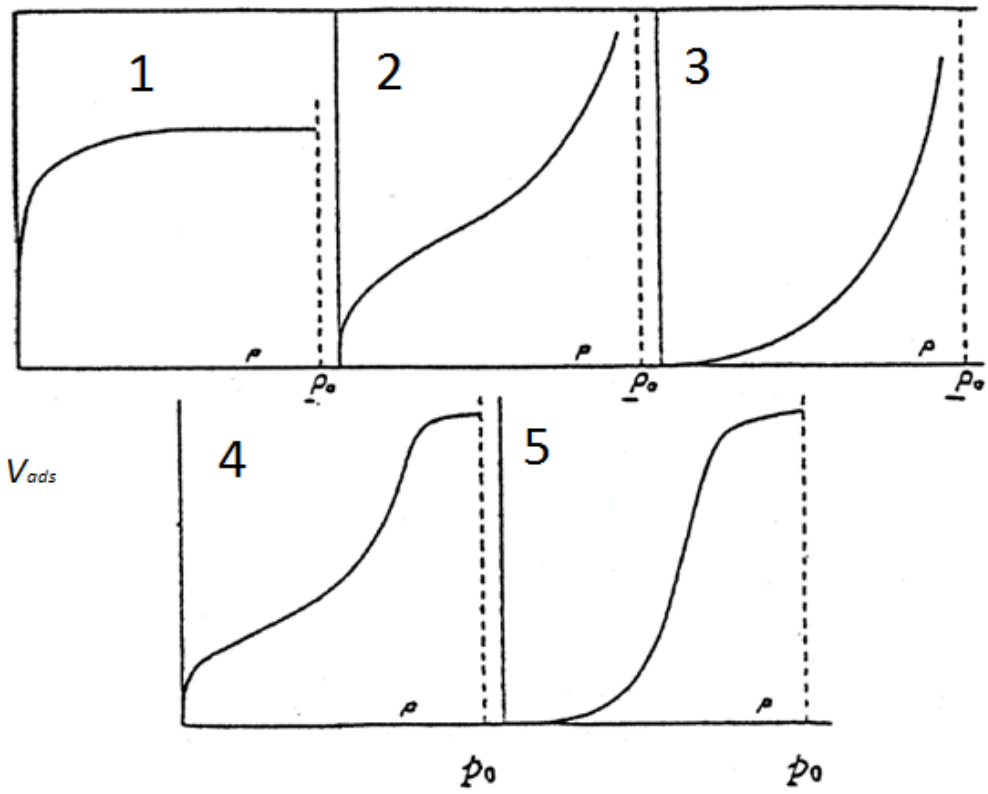

Figure 2. The five types of van der waals adsorption isotherms.
Representing the adsorbed concentration depending on the partial pressure of the given.component. According Brunauer et al. [72]

Liquid/solid equilibrium isotherms can be separated into four classes, those corresponding to ideal adsorption (the interactions between the bounded molecules are negligible. Hence it is not constructed another adsorbed layer above the adsorbed molecular layer, which happens instead for non-ideal adsorption) on homogeneous surface (only one type of adsorption center is known on the surface of the adsorbent), to ideal adsorption on heterogeneous surfaces (there are at least two independent binding sites with different adsorption energies on the adsorbent), to nonideal adsorption on homogeneous surfaces and to nonideal adsorption on heterogeneous surfaces.

The Langmuir isotherm is the most common model to describe nonlinear adsorption. And it describes ideal adsorption on homogeneous surfaces [73]. It is written as:

$$q = \frac{KC}{1+bC} = \frac{q_s bC}{1+bC} \qquad \text{Eq. 16}$$

where *C* and *q* are the concentration of the analyte in the mobile and the stationary phases, respectively. q_s- saturation capacity of adsorbent, *b* is the equilibrium constant or distribution coefficient.

For the modeling of ideal adsorption on heterogeneous surfaces bi-Langmuir, or the Tóth isotherm [74] are often used. Tóth model differs from the Langmuir isotherm only by an exponent. The role of exponent is to take into account the heterogeneity of the distribution of the sorption energies and given as:

$$q = \frac{q_s bC}{[1+(bC)^v]^{\frac{1}{v}}} \qquad \text{Eq. 17}$$

v is the heterogeneity parameter. It the value of the heterogeneity parameter is *v* = 1, than the Tóth isotherm equal with identical Langmuir isotherm. The smaller the value of *v*, the more disperse the energy distribution is. The Tóth isotherm corresponds to a unimodal adsorption energy distribution.

The biLangmuir isotherm model assumes the surface of stationary phase contains two different types of sites. This isotherm model is written as:

$$q = \frac{q_{s1} b_1 C}{1+b_1 C} + \frac{q_{s2} b_2 C}{1+b_2 C} \qquad \text{Eq. 18}$$

where q_{s1} and q_{s2} are the saturation capacities of two different types of sites and b_1 and b_2 are the equilibrium constants.

In case of multilayer adsorption an alternative model should be considered which is related to the names of Brunauer, Emmett and Teller (BET isotherm)

[75]. The equation of BET isotherm for the adsorption on homogeneous surfaces is written as:

$$q = \frac{q_s bC}{(1 - b_L C)(1 - b_L C + bC)} \qquad \text{Eq. 19}$$

where q_s is the monolayer saturation capacity, b and b_L are the equilibrium constants of the solute from the solution onto the bare surface of the adsorbent (b) and into a layer of adsorbate molecules (b_L). If there are no adsorbate – adsorbate interactions ($b_L = 0$) between two successive layers of adsorbate molecules, Eq. 19 reduces to the Langmuir isotherm. If b_L increases and the adsorbate – adsorbate interactions increase, the position of the inflection point shifts toward lower concentrations, until it reaches $C = 0$ and $b_L = b$ the isotherm becomes strictly antilangmuirian (type 3). The transition between types 2 and 3 of isotherms takes place when the second derivative of the isotherm becomes equal to 0 for $C = 0$.

3.3.2 Frontal Analysis method

Chromatography allows the choice among various approaches to determine adsorption isotherms. The most often used and the most accurate method to characterize the physico-chemical properties of chromatographic stationary phases is frontal analysis, which was first developed and used independently by James and Phillips [76] and by Schay and Székely [77]. Two main variations of the method exist to perform frontal analysis in liquid chromatography: the single step and the staircase (stepwise) technique (Figure 3).

The single step technique consists in replacing abruptly the mobile phase pumped into the column with a solution of the test compound, at a known, constant concentration in the same mobile phase and in recording the composition of the column eluate, determining the breakthrough curves. The test compound has to completely elute from the column and the column has to wash with pure mobile phase as long as necessary, after the breakthrough and equilibration. The principle of the staircase technique is to perform consecutive and abrupt concentration increases of the test compound in the eluent pumped

into the column and recording. The recording lasts for sufficient time to reach thermodynamic equilibrium between the mobile and stationary phases. This equilibrium is reached when a plateau concentration corresponding to the feed concentration is detected at the column exit.

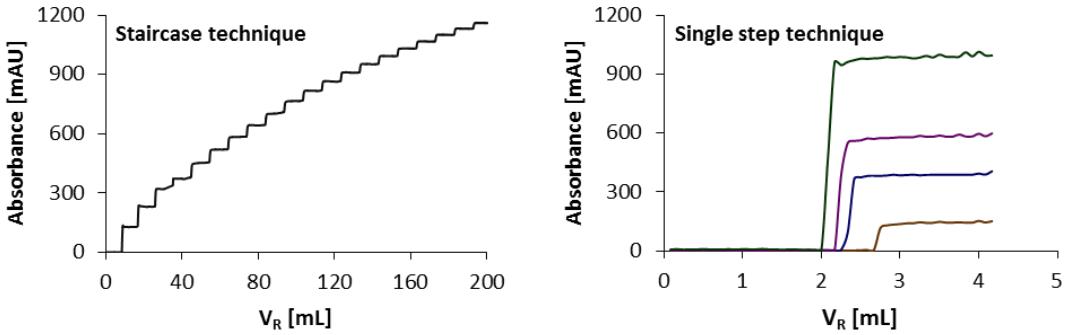

Figure 3. The single step and the staircase (stepwise) breakthrough curves in FA technique.

The breakthrough curves give the amount of the compound that is required to equilibrate the packing material in the column with the new solution thus from the breakthrough curves obtained, the values of stationary phase concentration, in equilibrium with the inlet concentration can be obtained through the following equation [78]:

$$q = \frac{(V_B - V_M)C}{V_{ads}} \qquad \text{Eq. 20}$$

where V_B is the retention volume of the self-sharpening shock (breakthrough volume); V_M is the system holdup volume and V_{ads} is the adsorbent material filling the column.

Eq. 21 assumes that the column was initially empty of solute and equilibrated with a stream of solution. A similar equation can be used if FA is carried out in the staircase mode. The integral mass balance of breakthrough curves between the equilibrium mobile phase concentrations C_i and C_{i+1} (in g/dm^3 of the solution) shows that the equilibrium concentration of the analyte on the stationary phase is given by [78]:

$$q_{i+1} = q_i + \frac{(V_{R,i+1} - V_D)(C_{i+1} - C_i)}{V_{ads}} \qquad \text{Eq. 21}$$

where q_i and q_{i+1} are the adsorbed concentrations of the analyte (in g/dm^3 of the adsorbent), when the stationary phase is in equilibrium with the solute mobile phase concentrations C_i and C_{i+1}, respectively, at the i^{th} and $(i + 1)^{th}$ step. $V_{R,i+1}$ is the retention volume of the breakthrough curve at the $(i + 1)^{th}$ step.

3.3.3 Inverse method

The main advantages of the inverse method over the most accurate frontal analysis are the lower cost due to the lesser solute consumption and reduced experimental time. The inverse method (IM) is a relatively recent numerical method for isotherm determination [79-86]. In 1991 Dose et al. used a modified simplex algorithm to obtain a better approach for the determination of equilibrium isotherms parameters of N-benzoyl-(D,L)-alanine and N-benzoyl-(D,L)-phenylalanine and found a good agreement with the isotherms resulted by frontal analysis [79]. In 1994 James and Sepúlveda developed a more sophisticated method to determine the best numerical estimates of parameters of an isotherm model from individual elution profiles of binary mixtures [80, 81] and Juza determined the isotherms of cycloheptanone and cyclopentanone by IM [82]. Antos et al. applied the IM for estimating the isotherm of methyl deoxycholate [83]. Moreover, the IM was applied a number of times for determining the numerical values of isotherm models of enantiomers, such as mandelic acid [84], 2,2,2-trifluoro-1-(9-anthryl)-ethanol [85], 1-indanol [86], 1-phenyl-1-propanol [87], Tröger's base [88], tryptophan [89] and ketoprofen enantiomers [90].

The macroscopic theory of the chromatographic separation process is based on the differential mass balance equations. The profiles of overloaded elution bands can be calculated – for instance – using the equilibrium-dispersive model (ED) that assumes constant equilibrium between the stationary and the mobile phases. In addition to adsorption and convection, all band-broadening transport effects are considered. Thereby, all kinetic effects are lumped together in the apparent dispersion coefficient. The following partial differential mass balance equation can be written as the basis of the model:

$$\frac{\partial C(z,t)}{\partial t} + \frac{V_S}{V_M}\frac{\partial q(z,t)}{\partial t} + u\frac{\partial C(z,t)}{\partial z} = D_a \frac{\partial^2 C(z,t)}{\partial z^2} \qquad \text{Eq. 22}$$

where z is the distance along the column, t the time and u the mobile phase linear velocity. D_a is the apparent axial dispersion coefficient that accounts for the dispersive phenomena (molecular and eddy diffusion) and for the non-equilibrium effects and it is estimated from the apparent number of theoretical plates:

$$D_a = \frac{HL}{2t_M} = \frac{Hu}{2} \qquad \text{Eq. 17}$$

where H is the height equivalent to a theoretical plate (HETP) for the component considered, t_M is the void time and L is the length of the column.

The initial conditions $C(z,0) = 0$ states that at $t = 0$ the stationary phase is in equilibrium with pure mobile phase. The boundary conditions which were used are the classical Danckwerts boundary conditions [91, 92] at the inlet and the outlet of the column. The concentration in the stationary phase is calculated as a function of the chosen isotherm model from the concentration in the mobile phase at any time. The ED model was solved using the modified Rouchon (finite difference) algorithm [93]. The inverse method estimates adsorption isotherm parameters by fitting simulated elution profiles to experimental ones. The determination of equilibrium isotherm parameters can be achieved through the following steps. First an overloaded elution band profile is recorded. Then an isotherm model is selected and initial estimates are given for its numerical parameters. Then the profile is calculated using the ED model of chromatography [91]. Finally, the calculated and recorded experimental profiles are compared by means of the following objective function:

$$\min \sum_i r_i^2 = \min \sum_i (C_i^{sim} - C_i^{meas})^2 \qquad \text{Eq. 18}$$

where C_i^{sim} and C_i^{meas} are the calculated and measured concentrations at point i and r_i is their difference. The isotherm parameters are changed to minimize the objective function, using a nonlinear least squares algorithm (the super modified downhill simplex search).

3.4 Determination of temperature dependence of the water adsorption in hydrophilic interaction liquid chromatography

Aqueous normal phase liquid chromatography had been used under different names for long time [94] before Alpert suggested the acronym HILIC. HILIC is an abbreviation form of hydrophilic interaction liquid chromatography, where the term “hydrophilic” implies the affinity for water [95]. Aqueous mobile phases were employed for the separation of carbohydrates and other compounds on polar stationary phases years ago [94, 96-98] and have been further elaborated by many later works. Already in the 1970s, Linden et al. applied the aqueous normal phase liquid chromatography technique for the separation of carbohydrates using amino-silica stationary phase and acetonitrile-water 75:25 (v/v%) mobile phase [94]. The interest in HILIC separations in the last decade has increased substantially for the separation of polar or ionized solutes including peptides, proteins, carbohydrates, oligosaccharides, drugs, metabolites and various natural compounds [99-101]. According to Alpert, polar solvents – especially water – strongly adsorb from mixed aqueous–organic mobile phases and form a diffuse water-rich layer at the surface of the adsorbent. In the HILIC mode, the retention is believed to be largely controlled by partition between the bulk mobile phase and the adsorbed water-rich layer.

However, because of the miscibility of polar organic solvents and water, no fixed boundary between the two liquid phases can be defined in the column. The retention may also be contributed by the adsorption of the solute onto the surface of a polar stationary phase [95].

HILIC separations employ a polar stationary phase and an aqueous–organic mobile phase, which usually contains acetonitrile and from less than 5% to 40% water, often with buffer additives adjusting the pH and ionic strength to reach stable retention [95, 102, 103].

Some elements of the mechanism in HILIC are similar to those in normal phase chromatography, involving hydrogen bonding and dipole-dipole interactions. Furthermore, the electrostatic interactions between charged solutes and hydrophilic stationary phases can also play important role in the HILIC

separation. Moreover, residual silanols may contribute to cation exchange and repulsion of anions, interactions that would be superimposed on partitioning and adsorption retention mechanisms. Silica with decreased surface concentration of silanol groups, such as hydrosilated silica [104, 105] was found suitable for the separation of polar compounds. Hydrosilated silica has more hydrophobic surface than ordinary silica, therefore hydrosilated silica can adsorb significantly less water than the ordinary silica as it was described by Soukup and his colleagues previously [106].

The adsorption of water on various silica-type columns and the comparison of the role of the different functionalities as well as the influence of temperature on the formation of the water enriched layer is a crucial problem. Soukup and his colleagues as well as Dinh and his colleagues [107, 108] suggested the determination of the adsorption isotherm of water on polar stationary phases by frontal analysis method and coulometric Karl-Fischer titration.

4 Experimental

4.1 Chemicals and analytes in case of cavitand experiments

Acetonitrile (ACN), methanol (MeOH) and water of gradient LC grade as well as thiourea (99%) were purchased from Sigma-Aldrich (Steinheim, Germany). The macrocyclic test compounds were C-tetra-methylcalix[4]resorcinarene (HRM); 2-methyl-C-tetra-methylcalix[4]resorcinarene (MRM); Cavitand (HCM) and methyl-cavitand (MCM) – shown in Figure 1 – were from the Inorganic Chemistry Department of University of Pécs.

4.2 Chemicals and analytes in case of water adsorption experiments

Acetonitrile, tetrahydrofuran (both gradient LC grade), thiourea (~99%), uracil, toluene, phenol and polystyrene standard (M_w = 1 800 000 g/mol) were purchased from Sigma-Aldrich (Steinheim, Germany). Using Karl Fischer titration, it was founded that the gradient LC grade acetonitrile did not contain any detectable amount of water. Water was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Coulomat AG, a reagent for coulometric Karl Fischer titration, was purchased from Sigma Aldrich.

4.3 Properties of columns in case of cavitand experiments

Manufacturers seek to increase the column efficiency and the area of application in different ways. The Hypersil BDS columns contain base-deactivated silica (Figure 4), which have some benefits such as reduced silanol interactions and consequently reduced peak tailing. The XTerra (Figure 4) columns are based on a combination of the most inert hybrid particles of Waters with the embedded polar group RP ligands [110]. These columns are generally less retentive than the regular C_8 and C_{18} counterparts for neutral and basic analytes due to “shielding” of the silanol groups by carbamate moieties. The

selectivity differences between a C_{18} and a polar-embedded phase column lead to very scattered correlation of their respective retention data.

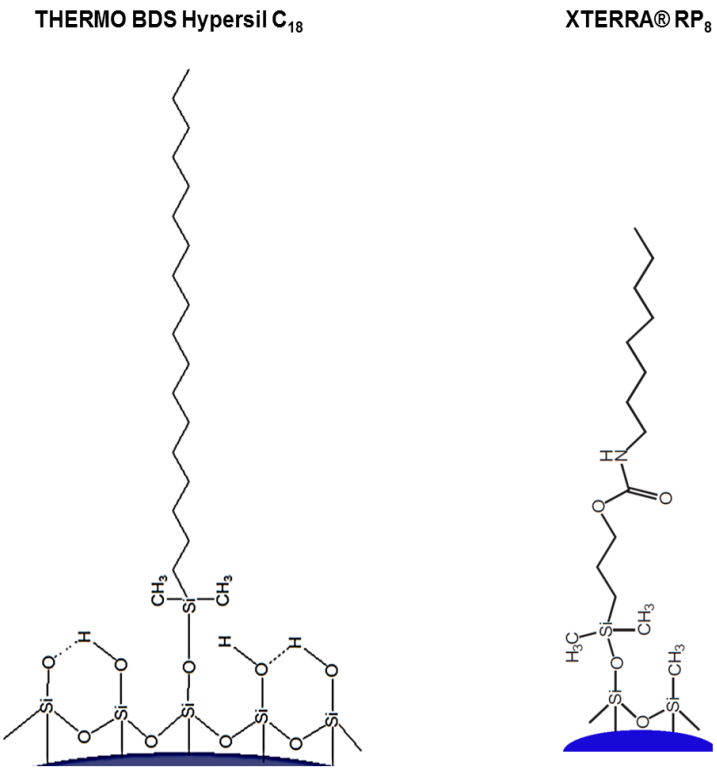

Figure 4. The Hypersil BDS C_{18} (contain base-deactivated silica) and the XTerra RP_8 (with the embedded polar group) columns

Antle et al. [111] showed that the differences in solute retention is correlated with the effective phase ratio of the column, the “polarity” of the bonded phase and the dispersion solubility parameter of the stationary phase. The phase ratio of the column is a function of the chain length, the bonding density, the surface area of the silica and the pore distribution. In reversed-phase chromatography the phase ratio strongly depends on the structure of the stationary phase. It is often assumed that the phase ratio is constant, more specifically, that the stationary phase volume is constant at different temperatures for homologous analytes [66].

The employed Hypersil BDS C_8 and C_{18} columns (100 × 4.6 mm; particle size 3 µm; pore size 120 Á) were obtained from Thermo Fisher Scientific, Inc. (Runcorn, UK) and the XTerra RP_8 and RP_{18} columns (100 × 4.6 mm; particle

size 3.5 µm; pore size 131 Á) were purchased from Waters Co. Ltd. (Saint Quentin en Yvelines, France). Zorbax C_{18} (50x2,1 mm)

The hold-up times were determined from injections of thiourea. For the calculation of the phase ratio we determined the volume of stationary phase (V_S) from the difference between the geometrical volume of the column and the void volume ($V_M = t_M F_v$).

4.4 Properties of columns in case of water adsorption experiments

In water adsorption investigation we used Cogent Silica C and Cogent Phenyl hydride columns (150 × 4.6 mm; particle size 4 µm; specific surface area 350 m^2/g). These were obtained from MicroSolv Technology Corporation (Eatontown, NJ, USA). According to manufacturer's data, both columns are based on a high-purity Type B silica backbone which is modified to cover the silica surface with silicon-hydride (Si-H) bonds, thus replacing >95% of surface silanols (Si-OH), but Cogent Silica-C has unbonded phase and the column stability temperature limit is 125 °C, while Cogent Phenyl hydride has hydrosilated silica surface with phenyl ligands. Temperature limite is 80 °C.

For each column, the hold-up volume (V_M) was measured as the elution volume of toluene in 100% tetrahydrofuran. The interstitial volume in between the particles (V_0) was determined as the elution volume of narrow-distribution high-molecular polystyrene standard with M_w = 1 800 000 in 100% tetrahydrofuran (as it was determined before studies on these columns [106, 107]). The total column porosity (ε_T) can be estimated as:

$$\varepsilon_T = \frac{V_M}{V_C} \qquad \text{Eq. 19}$$

where V_C (the volume of empty tube) is given by:

$$V_C = \pi r^2 L \qquad \text{Eq. 20}$$

For the 15 cm × 0.46 cm i.d. column (both investigated columns) V_C = 2.49 mL

The pore volume inside the column was calculated as the difference between the hold-up volume and the interstitial volume.

$$V_i = V_M - V_0 \qquad \text{Eq. 21}$$

4.5 Instruments and analysis conditions

4.5.1 Thermodynamic analysis conditions

The measurements were performed on an Agilent 1290 Infinity HPLC (Agilent Technologies, Waldbronn, Germany) system with a binary pump, a column oven and an autosampler. The instrument was equipped with a diode array UV-VIS detection system. Data acquisition, data handling and instrument control were performed with Agilent ChemStation Rev. B.04.03 software.

The isocratic elution was carried out with acetonitrile-water 55-45 (v/v%), methanol-water 72:28 (v/v%) and 88:12 (v/v%), the solvents were degassed by ultra-sonication before usage. The mobile phase was delivered at a flow-rate (F_v) of 1 mL/min. The experiments were carried out at 15, 25, 35 and 45°C and the precision of the temperature adjustment was ± 0.1°C. The injection volume was 2 µL and triplicate injections were applied. The detection was accomplished at 230 nm. The samples were dissolved in the mobile phase. The concentrations of all samples were 0.5 mg/mL. To study the logarithm of Henry constant versus volumetric fraction of organic modifier we determined the retention factors of HRM and MCM applying 65-96% methanol and 45-95% acetonitrile in the aqueous mobile phase.

4.5.2 The pressure induced retention changes analysis conditions

These measurements were also performed on the before mentioned Agilent 1290 Infinity HPLC system.

The isocratic elution was carried out with methanol:water 72:28 (v/v%) for HRM and 88:12 (v/v%) for MCM taking into account the solubility of the analytes. The

solvents were degassed by ultra-sonication before use. Mobile phase was delivered at a flow-rate of Fv= 0.1 mL/min. The column temperature was adjusted at 25 °C and the precision of the temperature adjustment was ± 0.1°C. The injection volume was 2 µL and triplicate injections were applied. The detection was accomplished at 230 nm. The samples were dissolved in the mobile phase. The concentration of each sample was 0.1 mg/mL. The hold-up times were determined from injections of thiourea. The experiments were carried out in the range of 1- 320 bar. The maximum applied pressure was limited by the pressure limitations the vendor specified for the columns. Column backpressure was increased by attaching capillary tubes of 30 µm ID and length from 5 to 15 cm. The tubes were attached between the end of the column and the detector using a zero-volume connector. Triplicate measurements of retention time were taken in each case and their average value was the basis of calculation. The average pressure ($p_{average}$) values were calculated from the inlet (p_{inlet}) and outlet (p_{outlet}) pressures:

$$p_{average} = \frac{p_{inlet} + p_{outlet}}{2} \qquad \text{Eq. 22}$$

The total pressure of the system (p_{total}) equated to the sum of the pressure before the column ($p_{pre\text{-}column} = p_{autosampler} + p_{tubing}$) + pressure of the column + pressure post column ($p_{post\text{-}column} = p_{restrictor} + p_{tubing} + p_{detector}$). The pressure at the column inlet is $p_{inlet} = p_{total} - p_{pre\text{-}column} = p_{column} + p_{post\text{-}column}$. The pressure at the column outlet is $p_{outlet} = p_{inlet} - p_{column} = p_{post\text{-}column}$. To summarize:

$$p_{average} = \frac{p_{column}}{2} + p_{post-column} \qquad \text{Eq. 23}$$

4.5.3 Overloading experiments

The overloaded band profiles were acquired using a Shimadzu HPLC system with two LC–10AD pumps and an SPD-M10A VP diode array UV-VIS detector. Data acquisition, data handling and instrument control were performed with the LabSolutions (Shimadzu) software.

The mobile phases were methanol-water 72:28 (v/v%) and 88:12 (v/v%) and were degassed by ultra-sonication before use. The measurements were carried out at 25 ^{o}C. Manual injector was used. The injection volume was 200 µL. The concentration of MCM was 0.6 g/L in methanol-water 88:12 (v/v%) and that of HRM was 3 g/L in methanol-water 72:28 (v/v%). The injection or inlet profile was estimated by replacing the column with a zero-volume connector and injecting directly into the detector.

The concentration profile of the components is derived from the original chromatograms (absorbance versus) through a calibration. Because the analytes have identical UV response factors, the detected absorbance signal of each compound can easily transformed into a concentration profile a single step:

$$C(t) = \frac{A(t)}{f} \qquad \text{Eq. 30}$$

The amount of the injected sample can be obtained by integrating the elution profile:

$$m = \int C(V)dV = F_v \int C(t)dt \qquad \text{Eq. 31}$$

In the range of linear detector response, absorbance is proportional to concentration, thus:

$$A(t) = fC(t) \qquad \text{Eq.32}$$

$$A_T = \int A(t)dt = f \int C(t)dt = \frac{fm}{F_v} \qquad \text{Eq.33}$$

When sensitivity *f* is determined by the above equation (linear detector response), the detector calibration is achieved:

$$f = \frac{A_T F_v}{m} \qquad \text{Eq. 34}$$

The inverse method calculations were made with an in-house created Fortran software. For the numerical integration of ED model a modified Rouchon (finite difference) algorithm was used [93].

4.5.4 Water adsorption experiments

All the water adsorption experiments were executed using an HPLC setup including a high-pressure pump (ECOM, Prague, Czech Republic) connected with a variable wavelength UV detector from the same manufacturer and an automated fraction collector (CF-1 Fraction Collector, Spectrum Chromatography, Houston, TX, USA). The detection wavelength was set to 210 nm. The chromatographic column was placed in a column oven compartment.

The temperature in the experiments was gradually increased up to the limits given by the column manufacturer: 40 °C, 60 °C, 80 °C, 100 °C in case of Cogent Silica C and 40 °C, 60 °C, 80 °C in case of the Cogent Phenyl hydride columns, respectively.

The concentration of water in the collected fractions was determined using an 831 KF Coulometer, a coulometric Karl Fischer titrator equipped with a 728 Ti Stand-magnetic stirrer (Metrohm, Herisau, Switzerland). An LC0101 (ECOM, Prague, Czech Republic) still air column thermostat was used at each experiment.

Before each measurement, the column was purged for at least 40 minutes with pure acetonitrile, in an effort to remove as much water as possible from silica. Then the column was disconnected from the system, the pump and the connecting capillaries were filled with the feed solution of water in acetonitrile. After that the column was again connected to the system and the isocratic pump was continuously delivering the feed solution onto the column at a constant flow rate (1 mL/min). The effluent passed through the column was collected in twelve fractions per minute.

The concentration of water in the collected effluent fractions was measured by coulometric Karl–Fischer titration method, which is the most commonly used

technique for accurate determination of water content at low levels. The determined values of water concentration were used to construct the breakthrough curves, the dependence of water concentration in each fraction on the volume of mobile phase passed through the column (Figure 16).

From the inflection points of the breakthrough curves, the breakthrough volumes (V_B), were evaluated and the excess concentration of water adsorbed on the stationary phase (q_{ex}) was calculated for each concentration of water in the mobile phase (C) as Eq. 35 shows:

$$q_{ex} = \frac{(V_B - V_M)C}{V_i} \qquad \text{Eq. 245}$$

where V_M is the column hold-up volume and V_i is the pore volume inside the column. The data were used for the construction of excess water distribution isotherms showing the dependence of concentration of excess water adsorbed on the stationary phase, on the concentration of water in the mobile phase. Both the concentration of excess water in the stationary phase and the concentration of water in the mobile phase are expressed as volume fraction of water.

5 Results and discussion

5.1 Determination of retention behavior of the cavitands by linear chromatography

5.1.1 Influence of temperature on the retention of resorcinarene-based cavitands

In this part I present the effect of the mobile phase on the retention of cyclic oligomers *via* the elution of resorcinarenes and cavitands under isocratic conditions on C_8 and C_{18} stationary phases from two manufacturers at 25 °C. The measurements were carried out by using acetonitrile-water 55:45 (v/v%) and methanol-water 72:28 (v/v%) eluents. We chose the ratio of the latter mixture to get similar retention value in methanol- and in acetonitrile-containing mobile phases. The acetonitrile-water 50:50 (v/v%) and methanol-water 70:30 (v/v%) mobile phases are virtually equieluotropic for homologous analytes [109] so methanol-water 72:28 (v/v%) mobile phase was applied. In each chromatogram, only the main peak was considered.

The Figure 5, shows huge difference between the retention of resorcinarenes and cavitands. While the retention time (t_R) of resorcinarenes was in the range of t_R = 2-6 min, for HCM it was t_R = 16-40 min and for MCM it values were between t_R = 37-145 min on the four columns when the same mobile phase composition (acetonitrile-water 55:45 v/v%) was used. Surprisingly, the retention time of cavitands was even a hundred times larger than the retention time of resorcinarenes. Besides – within a family of compounds – the presence of methyl groups on the upper rim in the molecules caused a large increase of the retention on each column. The retention times were almost 2 to 3 times larger for MRM than for HRM (Figures 5 a, c, e, g) and the retention times of MCM were 2 to 4 times larger than those for HCM (Figures 5 b, d, f, h).

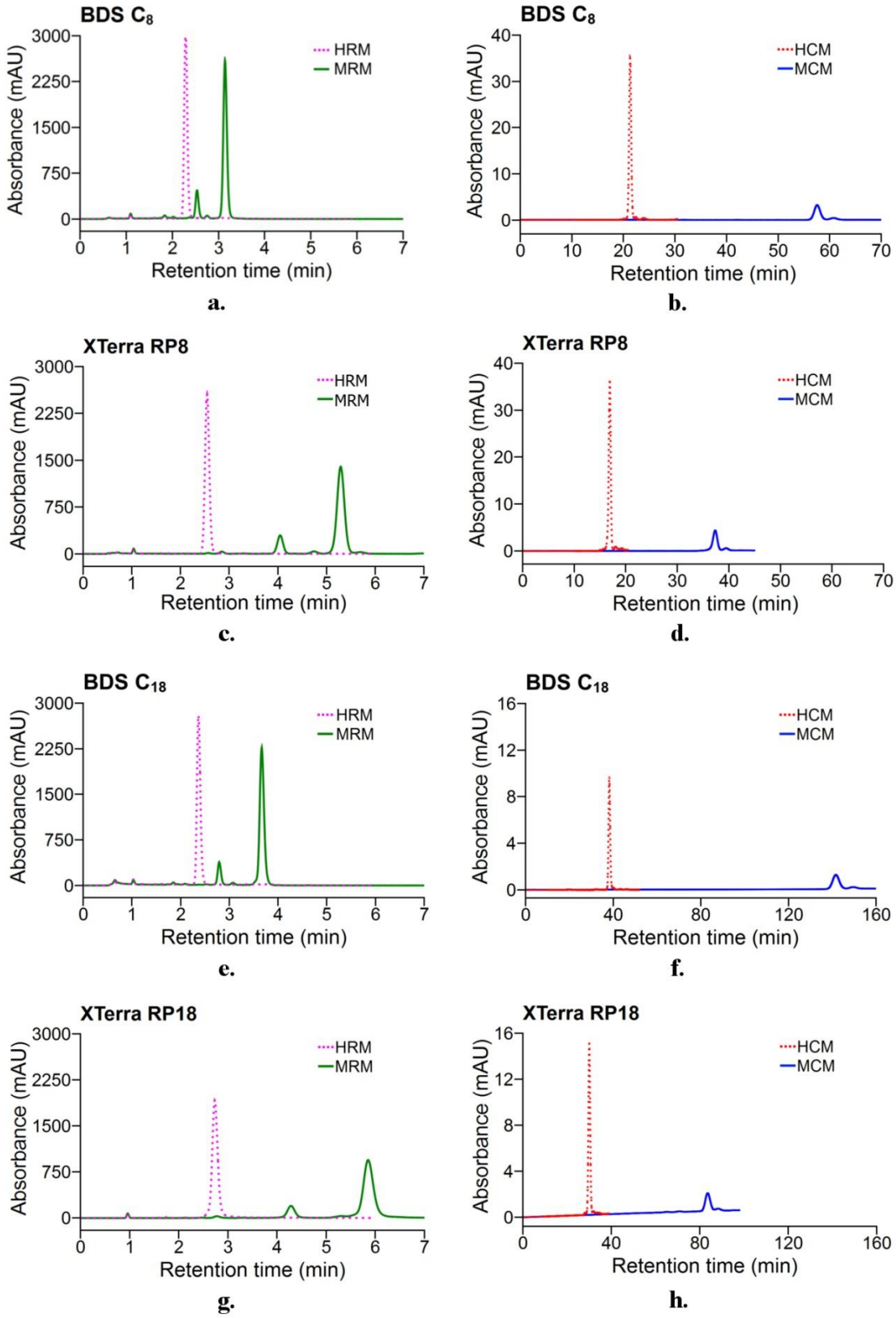

Figure 5. Chromatograms of HRM, MRM, HCM and MCM on the C_8 and C_{18} stationary phases (25 °C, 230 nm, mobile phase 55:45 = ACN:H_2O v/v%).

The above-mentioned wide range of retention times for each analyte suggests that there is a large difference between the retention behaviors of a solute on the four columns. Comparing the two types of stationary phases, on the XTerra RP_8 column the retention of resorcinarenes was larger (Figure 5 c) than that on the BDS Hypersil C_8 column (Figure 5 a). However, the retention times of cavitands (HCM, MCM) were smaller on the XTerra RP_8 column (Figure 5 d) than on the BDS Hypersil C_8 column (Figure 5 b). We could observe the same phenomenon on the C_{18} stationary phases. On the XTerra RP_{18} column, the retention of resorcinarenes was larger (Figure 5 g) than on the BDS Hypersil C_{18} column (Figure 5 e). However, the retention times of cavitands were smaller on the XTerra RP_{18} column (Figure 5 h) than on the BDS Hypersil C_{18} column (Figure 5 f). The opposite retention behavior of the resorcinarenes and cavitands on the two types of stationary phases showed well the difference of the selectivity of the XTerra and BDS Hypersil columns.

The effect of the chain length of the bonded stationary phase could be observed in the case of cavitands. The retention time was at least twice larger on the C_{18} columns (XTerra and BDS Hypersil) than on the C_8 columns (Figures 5 b, f and d, h). The retention times of the resorcinarenes on the two columns (C_8 and C_{18}) were within one minute difference (Figures 5 a, e and c, g). Consequently, the apolar chain length has lesser influence on the retention of resorcinarenes (HRM, MRM) than .the retention of cavitands (HCM, MCM).

The above observations were in consequence of the combined effect of the solute structure, the stationary phase properties, the mobile phase composition and the different interactions between the solute and chromatographic phases on given temperature. To understand the retention mechanism, the chromatograms were recorded at different temperatures on every column, thus, the van't Hoff plots, i.e. the logarithm of retention factor (ln k) against the reciprocal of temperature (1/T) could be generated. In acetonitrile-water system, the calculated van't Hoff plots represented Figure 6. In both cases (acetonitrile-water and methanol-water system) the plots showed good linearity over the temperature range investigated (15 – 45 °C). The correlation coefficient (R^2) for

the linear fit of the plots ranged from 0.9987–0.9997, 0.9978–1.000, 0.9998–1.000 and 0.9998–1.000 for the HRM, MRM, HCM and MCM, respectively.

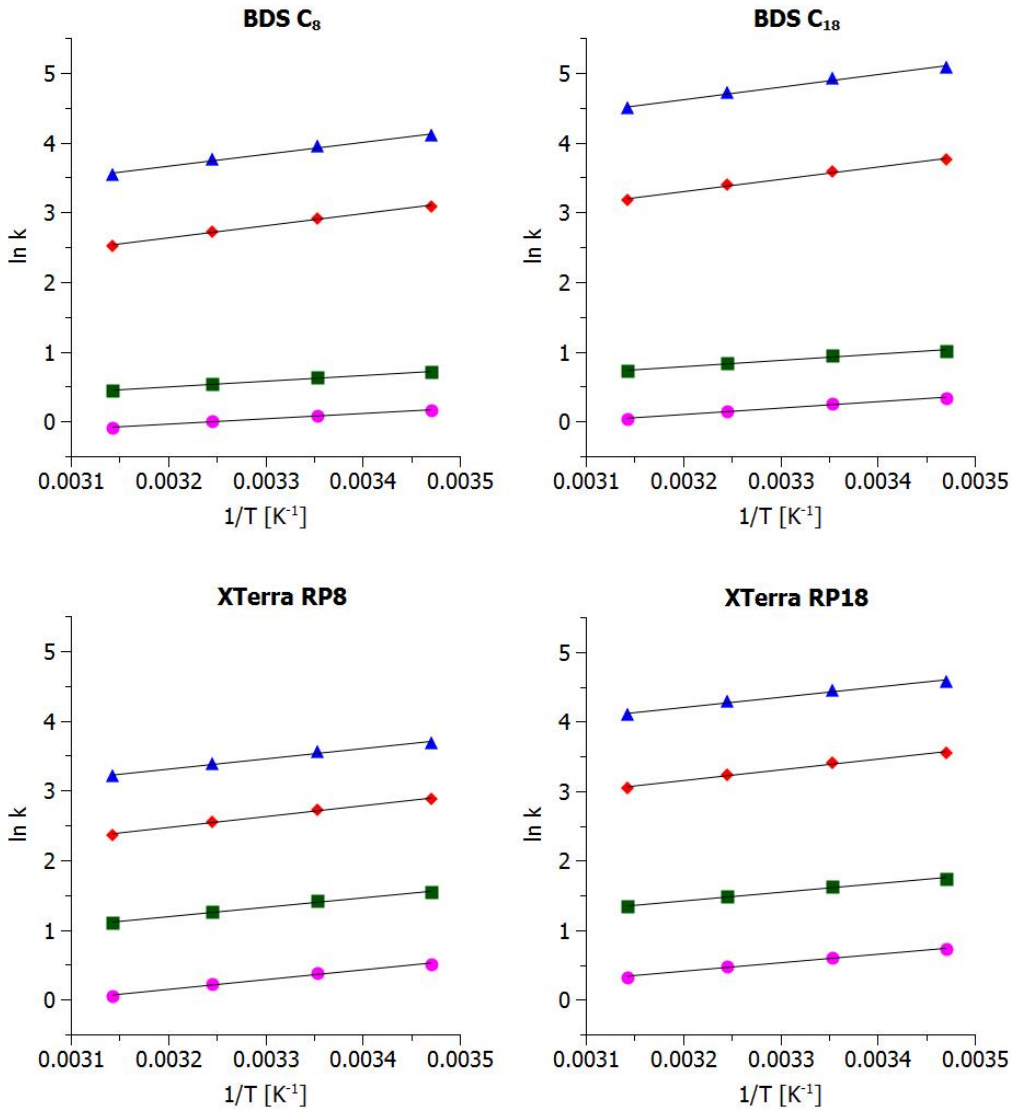

Figure 6. The calculated van't Hoff plots of HRM (●), MRM (■), HCM (♦) and MCM (▲) on the C_8 and C_{18} stationary phases (mobile phase 55:45 ACN:H_2O v/v%).

The retention factors and thermodynamic parameters of analytes are presented in Table 1. By comparing the retention factors of the analytes in two types of mobile phases discernible that the retention factors of resorcinarenes were approximately twice larger in methanol-water than in acetonitrile-water eluent (as it can be seen in Table 1). By contrast, the retention factors of cavitands were smaller in methanol-containing mobile phase than in acetonitrile-containing eluent. Summarizing, the retention factors of the analytes were

closer to each other in methanol-water than in acetonitrile-water mobile phase on each column.

Table 1. Retention factors and thermodynamic parameters of HRM, MRM, HCM and MCM in (A) methanol-water 72:28 (v/v%) and (B) acetonitrile-water 55:45 (v/v%) mobile phases (TΔS values are calculated for 303 K). [7, 8]

A

MeOH/H_2O 72:28 v/v%	**C_8**				**C_{18}**			
	HRM	MRM	HCM	MCM	HRM	MRM	HCM	MCM
XTerra								
k	1.90	5.79	8.63	26.65	3.64	9.35	19.25	75.04
ΔS (J K^{-1} mol^{-1})	-61.29	-70.09	-52.62	-57.05	-68.13	-74.8	-54.28	-58.17
ΔH (kJ mol^{-1})	**-21.93**	**-27.33**	**-23.10**	**-27.20**	**-25.1**	**-29.43**	**-25.1**	**-29.64**
TΔS (kJ mol^{-1})	-18.58	-21.25	-15.95	-17.29	-20.65	-22.68	-16.46	-17.63
ΔG (kJ mol^{-1})	-3.35	-6.08	-7.14	-9.90	-4.45	-6.76	-8.65	-12.01
BDS								
k	2.25	3.00	9.17	33.22	4.27	6.42	24.69	121.46
ΔS (J K^{-1} mol^{-1})	-76.11	-79.31	-57.7	-63.02	-82.34	-81.16	-58.9	-62.38
ΔH (kJ mol^{-1})	**-27.07**	**-28.74**	**-25.06**	**-29.84**	**-30.09**	**-30.74**	**-27.45**	**-32.43**
TΔS (kJ mol^{-1})	-23.07	-24.04	-17.49	-19.10	-24.96	-24.6	-17.85	-18.91
ΔG (kJ mol^{-1})	-3.99	-4.70	-7.57	-10.74	-5.13	-6.14	-9.59	-13.52

B

ACN/H_2O 55:45 v/v%	**C_8**				**C_{18}**			
	HRM	MRM	HCM	MCM	HRM	MRM	HCM	MCM
XTerra								
k	1.35	3.82	14.09	32.18	1.72	4.75	27.82	78.88
ΔS (J K^{-1} mol^{-1})	-31.89	-21.84	-16.81	-7.52	-26.43	-18.85	-12.2	-1.65
ΔH (kJ mol^{-1})	**-11.65**	**-11.22**	**-12.98**	**-12.25**	**-10.15**	**-10.4**	**-12.82**	**-12.25**
TΔS (kJ mol^{-1})	-9.67	-6.62	-5.09	-2.28	-8.01	-5.71	-3.7	-0.5
ΔG (kJ mol^{-1})	-1.99	-4.6	-7.89	-9.97	-2.13	-4.68	-9.12	-11.76
BDS								
k	1.04	1.79	16.99	47.29	1.22	2.42	33.07	124.23
ΔS (J K^{-1} mol^{-1})	-15.21	-12.24	-18.92	-9.65	-19.67	-13.5	-15.54	-5.68
ΔH (kJ mol^{-1})	**-6.34**	**-6.8**	**-14.45**	**-14.22**	**-7.66**	**-7.51**	**-14.67**	**-15.01**
TΔS (kJ mol^{-1})	-4.61	-3.71	-5.74	-2.93	-5.96	-4.09	-4.71	-1.72
ΔG (kJ mol^{-1})	-1.73	-3.09	-8.71	-11.29	-1.69	-3.41	-9.96	-13.29

The signs and magnitudes of enthalpy, entropy and Gibbs free energy changes of the analytes could be obtained from the plots which provide useful information on the retention process. The calculated values of the enthalpy

changes reflect that the transfer of the solute from the mobile phase to the stationary phase is usually enthalpy controlled process (as usually in RPLC). In acetonitrile-water system the values of the enthalpy change of the analytes ranged from ΔH = –10.20 kJ/mol to –15.0 kJ/mol on every column, except for the enthalpy change of the resorcinarenes on the BDS C_8 and C_{18} columns (ΔH = –6.30 to –7.7 kJ/mol). The methylated cavitand (MCM) has the most negative enthalpy change on the BDS Hypersil C_{18} column (ΔH = –15.0 kJ/mol) and the demethylated resorcinarene (HRM) has the least negative enthalpy change on the BDS Hypersil C_8 column (ΔH = –6.3 kJ/mol).

The enthalpy change of the analytes in methanol – water system ranged from ΔH = –21.90 to –32.4 kJ/mol on every column. ΔH = –32.4 kJ/mol is the enthalpy change of MCM on the BDS C_{18} column. It is the most negative enthalpy change and the HRM has the least negative enthalpy change on the XTerra RP_8 column (ΔH = –21.9 kJ/mol). Miyabe et al. [106] also observed that the absolute value of the enthalpy change was smaller in acetonitrile-water than in methanol-water system and concluded the interactions between the octadecyl ligands and solutes were weaker in acetonitrile-water than methanol-water system.

It can be ascertained that if the organic solvent in the mobile phase is replaced by methanol, the enthalpy change values become more negative which means that the interactions between the analytes and stationary phases are favorable, although the entropic contribution reflects that the interactions become more unfavorable compared to the thermodynamic parameters measured in acetonitrile-water mobile phase. In acetonitrile-water system, the values of the product of the entropy changes and temperature ($T\Delta S$) for the analytes are in the range of –0.5 to – 9.67 kJ/mol, while in methanol-water mobile phase in a range of –16.0 to –25.0 kJ/mol on the different stationary phases. The product of entropy change and temperature is the least negative –0.5 to –2.9 kJ/mol for MCM on every column among the tetramers in acetonitrile-water system. This means that the change of the entropy of MCM is smaller compared to that of the other analytes. So in acetonitrile-water system, on every column, the entropic contributions of solutes containing methyl groups on the upper rim are more than that of the analytes containing hydrogen atom on the upper rim.

In context with the retention factors, the Gibbs free energy changes of the analytes were in the range of −1.7 to −13.3 kJ/mol with both types of eluents on the different stationary phases. The value of the Gibbs free energy change of MCM is the most negative (ΔG = –9.9 to –13.3 kJ/mol) on every column among the solutes.

The adsorption of the eluent on the stationary phase surface and the solvation phenomena have a major impact in the interpretation of the retention mechanism. When the organic modifier is replaced by another one, the properties of the stationary phase surface also change. The composition of the mobile phase at the surface is different from its bulk composition. The less polar solvent components of the mobile phase could accumulate near the apolar surface of the stationary phase, forming an essentially stagnant layer of the mobile phase, which is rich in the less polar solvent.

Many publications have studied the solvent excess adsorption on different types of stationary phases with varying coverage density [112-114]. The general conclusion of those studies is that the adsorbed layer thickness of acetonitrile appears to be approximately four or five layers of acetonitrile molecules. The thick acetonitrile layer adsorbed on the bonded phase surface causes the analyte partitioning into this adsorbed layer, which has a role in the retention mechanism, too. However, the adsorbed layer thickness of methanol on the same adsorbents is roughly equivalent to the monolayer-type adsorption. The thickness of the adsorbed layer is largely independent of the length of underlying bonded alkyl chains; therefore, there is no essential difference between the adsorbent layers of organic modifiers on C_8 and C_{18} reversed-phase columns. Bocian et al. observed that the acetontitrile adsorption is about four times larger than that of methanol for C_{18}, phenyl, cholesteryl, cyano, amido (polar embedded) stationary phases [114].

Therefore, we consider the thickness of the adsorbed layer comparable in the same type of stationary phases containing alkyl or polar embedded alkyl groups. The adsorbed methanol can provide hydroxyl groups to create hydrogen bonds with the solvated analytes, so the values of the enthalpy changes correspond to the strength of the hydrogen bonds as intermolecular

forces (20–30 kJ/mol) [115]. This is the reason why the enthalpy changes of resorcinarenes and cavitands are roughly 2–4 times larger in methanol than in quasi equieluotropic acetonitrile containing eluent. In methanol-water mobile phase, the enthalpy changes of resorcinarenes became comparable on BDS Hypersil and XTerra stationary phases, because the role of the polar embedded groups does not appear in methanolic eluent.

The dispersive interactions are in the range of 5–20 kJ/mol, this is equal to the enthalpy changes of the analytes in acetonitrile-water mobile phase because the adsorbed acetonitrile can offer four π-electrons to create dispersive interactions with the analyte molecules. In acetonitrile-containing eluent, the effect of polar embedded groups of the XTerra column can manifest, because of the creation strong hydrogen bonds between the polar carbamate groups and the hydroxyl groups of resorcinarenes. In addition to the above mentioned interactions the formation of the -C-H…π bonds between the examined analytes containing aryl rings and the alkyl chains of the stationary phase also contributes to the value of enthalpy change, which is favorable on the octadecyl bonded silica.

5.1.2 Influence of pressure on the retention of resorcinarene-based cavitands

The results showed that the retention factors of the resorcinarene and cavitand increased with the increase of the pressure. According to Eq. 13, the plots of ln *k* versus average column pressure measured on each column showed good linearity over the pressure range investigated, as we can see in Figure 7.

The slope, intercept and correlation coefficient of the plots are represented in Table 2. As we mentioned earlier, if the phase ratio remains constant, the molar volume change of the analyte could be derived directly from the slope ($-\Delta V_m/RT$) of the plots.

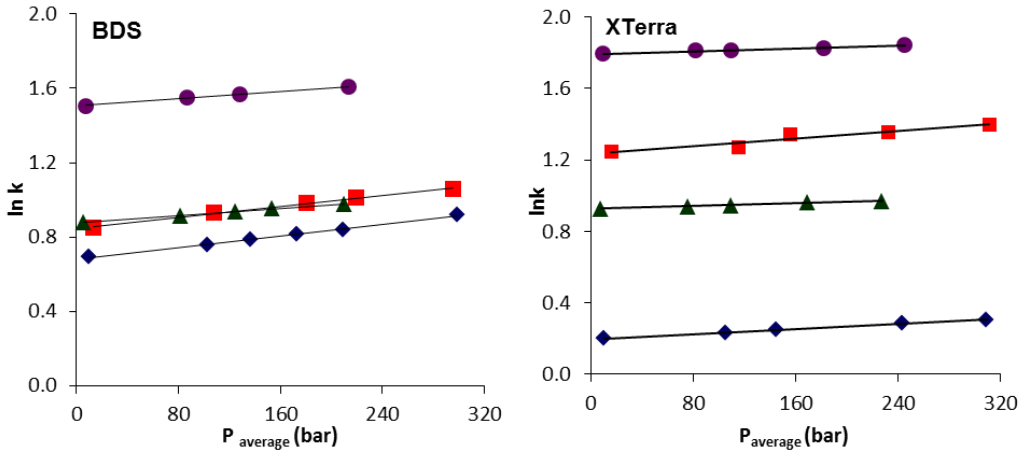

Figure 7. Logarithm of retention factor (ln k) as a function of average pressure (T = 303 K). The analytes and columns (Hypersil BDS and XTerra) are (♦)HRM, C_8; (■) HRM, C_{18}; (▲) MCM, C_8 and (●) MCM, C_{18}

The calculated change of molar volume of the HRM and MCM are summarized in Table 2. The changes of molar volume of the analytes are negative in every case due to the reduction of their solvation shell and some conformational change during adsorption. We can observe that the molar volume change of HRM is approximately 1.5 times larger than that of MCM, regardless of the type of stationary phase.

Table 2. Slope, intercept and correlation coefficient (R^2) of ln *k* of HRM and MCM versus the average pressure and The molar volume change of HRM and MCM calculated from the slope ($-\Delta V_m/RT$) based of Eq. 13 at 303 K).

HRM	Slope (10^{-4})	Intercept	R^2	ΔV_m (mL/mol)
BDS C_8	7.728	0.682	0.997	-19.16
BDS C_{18}	7.36	0.846	0.993	-18.24
XTerra RP_8	3.517	0.198	0.994	-8.72
XTerra RP_{18}	5.24	1.234	0.911	-12.99
Zorbax C_{18}	6.98	1.908	0.996	-17.3
MCM	Slope (10^{-4})	intercept	R^2	ΔV_m (mL/mol)
BDS C_8	4.859	0.877	0.994	-12.04
BDS C_{18}	4.751	1.506	0.992	-11.78
XTerra RP_8	1.96	0.926	0.992	-4.86
XTerra RP_{18}	1.983	1.793	0.982	-4.92

The more polar, conformationally flexible HRM molecule which contains eight hydroxyl groups can adopt more solvent molecules (methanol/water) in the mobile phase than the apolar MCM, so the molar volume change of resorcinarene and in this context the desolvation is larger than that of cavitand, when it binds to the apolar stationary phase. 72 and 88 % methanol was used as mobile phase modifier for HRM and MCM, respectively. In the presence of this relative high organic content the alkyl chains begin to expand significantly, so there is a large contact area for hydrophobic interaction with the analyte. Li and his colleagues [116] studied the role of the solvation shell decomposition of the alkali metal ions in their complexation by resorcinarene and its cavitand in methanol. They observed that the host-guest interaction with the resorcinarene is enthalpy-controlled and the complex formation with cavitand is driven by entropy.

The flexibility of the resorcinarene reduced significantly during the complexation. The cations were solvated when they bind *via* hydrogen bonds to the resorcinarene but they were unsolvated when they create cation –π interactions with the cavitands. When the cyclic tetramers interact with the hydrophobic ligands of the stationary phase, the desolvation process of the resorcinarenes and cavitands must be contrary to the interaction with polar ions as the results of our experiments confirm that. If we compare the change of molar volume of the analytes obtained on the BDS Hypersil and XTerra columns, the molar volume change of HRM and MCM is twice larger in absolute terms on the octadecyl bonded than it is on the polar-embedded stationary phase as we accepted.

Rafferty et al. [117] examined the effect of embedded polar groups and pressure on the structure of C_{18} stationary phase with Monte Carlo simulations. They found that there is an increased amount of solvent in the polar-embedded phase and the pressure-induced changes of octadecyl phase structure were small between 1–400 bar. The previous work of Rafferty et al. [118] gives more details about the structural difference of polar-embedded and C_{18} stationary phases. According to the solvent density profiles of amide polar-embedded phase, there was a maximum in the solvent density at about 9 Å from the silica

surface corresponding to the position of the amide group. The solvent density profiles of the C_{18} phase was close to zero in an extend region (between 6 and 12 Å) because the carbon density was close to the bulk density for liquid n-octadecane. The embedded polar groups can create hydrogen bond interactions directly with other embedded polar groups and solvent molecules or *via* solvent tethers with embedded polar groups and silanols.

Consequently, the polar-embedded phase is much more wettable and more ordered than the C_{18} phase. Martire et al. [119] described that the retention mechanism in methanol-water mobile phase is governed by solvent – solvent hydrogen bonding. Thus, the solvent-enriched polar-embedded phase can create polar interactions with the solvated analytes.

Presumably, the solvated molecules can bind to the polar-embedded stationary phase with a slight loss of their solvation shell but the binding to the hydrophobic, octadecyl bounded stationary phase needs a larger loss of their solvation layer.

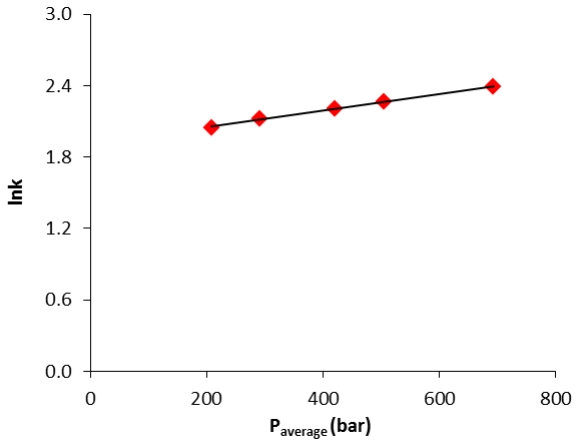

Figure 8. Logarithm of retention factor (ln k) of HRM on Zorbax C_{18} column as a function of average pressure (T = 303 K).

These results were confirmed when the pressure was increased to 700 bar applying an UHPLC C_{18} column (Figure 8). The Hypersyl BDS and the XTerra columns cannot be used beyond 320 bar pressure drop, therefore a Zorbax UHPLC column was used for reference measurements. The calculated molar volume change of HRM on Zorbax C_{18} is in good agreement with the result obtained for HRM on BDS Hypersil C_{18}, as we can see in Table 2 regardless the

range of pressure. A comparison of the C_8 and C_{18} of the stationary phases shows that the molar volume changes of the analytes are independent of the chain length. Melvin et al. [70] could observe differences between the octyl- and octadecyl-bonded silica only in some analytes, not in every case. Although the analytes bind stronger to the C_{18} than to the C_8 phases [8], sometimes the size of the solvation shell has no influence on that.

By knowing the geometric parameters of the resorcinarene-based cavitands, we can estimate the change of the volume for one molecule. The average diameter of resorcinarene and cavitand [120, 121] is about 7 Å between the opposite aromatic ring centroids. Probably the molecules are cylindrical structures and the height of the cylinder is about 4 Å. The estimated volume of one molecule is around 150 $Å^3$. If we divide the molar volume change of the analyte (Table 2) with the Avogadro constant, the volume change of one molecule can be obtained. The calculated change of volume of HRM is 30 $Å^3$ on the BDS Hypersil and 15 $Å^3$ on the XTerra columns, which values represent 20 and 10% of the total volume of the unsolvated molecule, respectively. The calculated volume change of MCM is 20 $Å^3$ (13%) on the BDS Hypersil and 8 $Å^3$ (5%) on the XTerra columns. Although this rough approximation does not consider the size of the solvation shell around the molecule, it can shed light on the degree of the desolvation when the molecule binds to the stationary phase. Because of the presence of solvation shell in the mobile phase, the relative volume change of one molecule may be less than the calculated values. Presumably, the volume change of the resorcinarene and cavitand molecules is only a few percent of the total volume of the solvated molecule.

5.2 Determination of retention behavior of the cavitands by nonlinear chromatography

5.2.1 Determination of the adsorption isotherm parameters using the inverse method

The retention factor and the elution profile of a solute depend on its ability to adsorb to the stationary phase surface and on how it competes with other components. Adsorption isotherms describe the distribution of the solute

between the mobile and stationary phases. For the determination of the adsorption isotherms, we chose the inverse method because the solubility of HRM and MCM was so limited in the mobile phase. Moreover applying frontal analysis needed bigger amount of HRM and MCM than the available amount of these. The inverse method requires the determination of the inlet concentration profile because the extra-column dispersion increases the spread of the recorded band profile and it constitutes the boundary condition of differential mass balance equation. In an ideal case the inlet concentration profile has a rectangular form [122] but in practice significant deviations are observed from this profile as we can see in Figure 9.

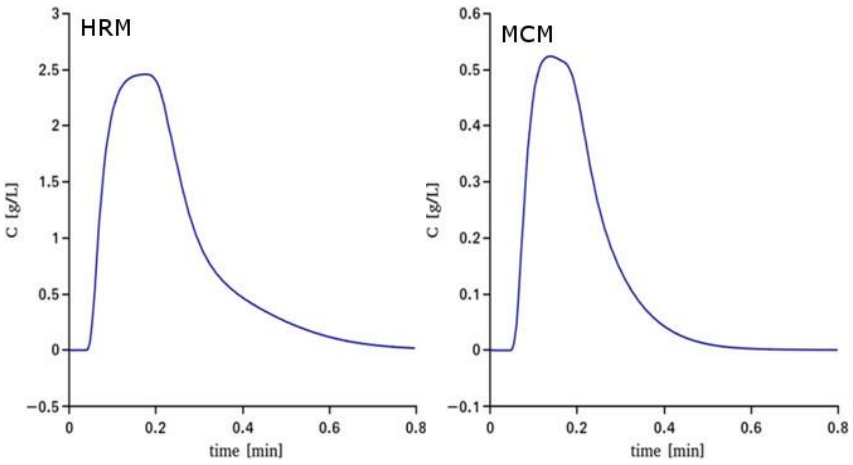

Figure 9. Plot of the inlet concentration profile of HRM and MCM

Overloaded band profiles were recorded by injecting 0.12 mg MCM and 0.6 mg HRM in saturated solutions. Due to the limited analyte solubility the isotherms could be determined only slightly beyond the linear range, the obvious choice to model the adsorption behavior seemed to be the Langmuir isotherm.

Using the inverse method, firstly the K parameter of Langmuir isotherm was estimated which could be determined according to Eq. 38 as we mentioned above. At this point, the least-squares estimation of *b* did not present any difficulty. Then the isotherm parameters (*K* and *b*) were tuned so that the calculated and the measured band profiles match as much as possible.

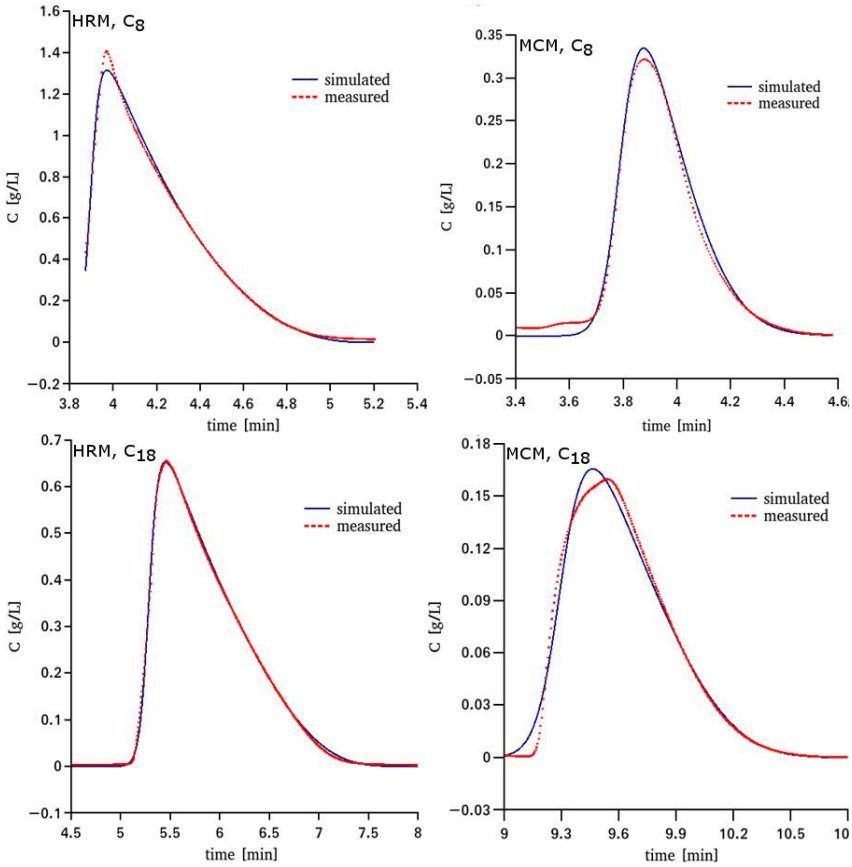

Figure 10. The (—) simulated and (…) measured chromatograms of HRM and MCM on Hypersil BDS columns. The analytes and columns are (A) HRM, C_8; (B) MCM, C_8; (C) HRM, C_{18} and (D) MCM, C_{18}

The best-fit chromatograms are plotted in Figure 10 and the numerical results are summarized in Table 7. The best value of the final sum of squares of residuals (FSSR is the variance of fitting errors) was achieved in the case of MCM on C_{18} column (FSSR = 0.01181) and the worst fitting was obtained by HRM on C_8 column (FSSR = 0.1242). Although due to the rather small amount of the analytes, the isotherm data were nearly linear over the examined range of concentration, we found that the Langmuir isotherm model represents well the experimental data. In Figure 11 we can see the Langmuir isotherms determined with the inverse method.

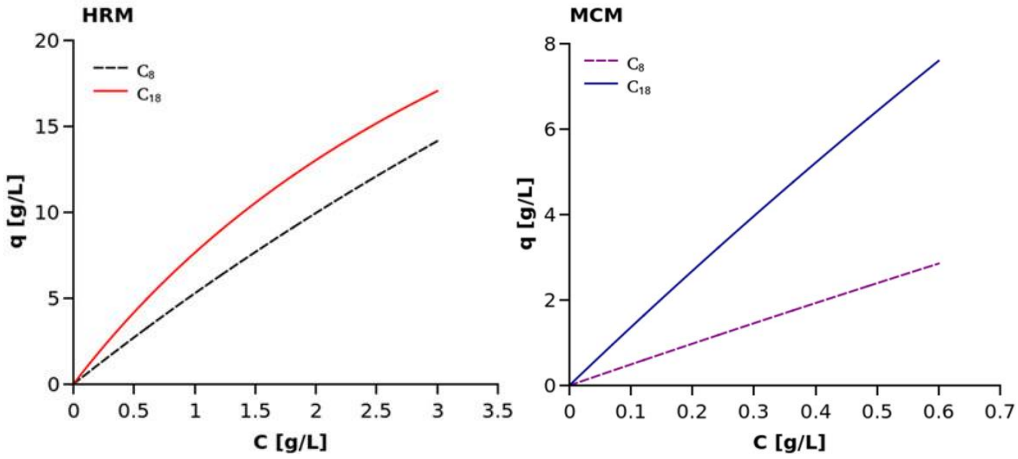

Figure 11. Langmuir isotherms determined by IM. The analytes and Hypersil BDS columns are HRM on C_8 and C_{18} and MCM on C_8 and C_{18}

Table 7. Langmuir isotherms parameters determined by the inverse method and the influences of the random errors (n = 0.01, 0.02 and 0.05) on the Langmuir isotherm parameters (*b, q_s*); diff. % - shows the absolute percentage differences between the isotherm parameters determined by IM and the isotherm parameters with random errors.

HRM		IM	n=0.01	diff.%	n=0.02	diff.%	n=0.05	diff.%
C_8	b	0.064	0.064	0	0.065	1.54	0.0678	5.6
	q_s	87.8	86.87	1.07	85.96	2.14	83.36	5.33
	FSSR	0.124						
	R^2		0.999		0.999		0.994	
C_{18}	b	0.208	0.208	0	0.209	0.48	0.21	0.95
	q_s	44.39	44.31	0.18	44.23	0.36	43.98	0.93
	FSSR	0.033						
	R^2		0.999		0.9987		0.9919	
MCM		IM	n=0.01	diff.%	n=0.02	diff.%	n=0.05	diff.%
C_8	b	0.0718	0.076	5.53	0.079	9.11	0.092	22
	q_s	69.12	65.5	5.53	62.26	11	54.25	27.4
	FSSR	0.03						
	R^2		0.999		0.999		0.995	
C_{18}	b	0.154	0.158	2.53	0.161	4.35	0.174	11.5
	q_s	90	87.83	2.47	85.78	4.92	80.18	12.3
	FSSR	0.012						
	R^2		0.9998		0.9991		0.994	

The adsorption–desorption equilibrium constant for HRM was three times larger on the C_{18} (b = 0.2079 L/g) than on the C_8 column (b = 0.0640 L/g). The adsorption – desorption equilibrium constant of MCM was found twice larger on the C_{18} (b = 0.1537 L/g) than on the C_8 column (b = 0.0718 L/g).

These values of the b isotherm parameter suggest that the adsorption energy of HRM is 2.87 kJ/mol larger and the adsorption energy of MCM is 1.85 kJ/mol larger on the C_{18} than on the C_8 BDS Hypersil column. The saturation capacity of HRM on the C_8 column was twice larger than that on the C_{18} column (Table 7)

The results show that on the octadecyl bonded silica there is a smaller number and stronger binding sites for HRM while on the stationary phase containing octyl ligands, there is a larger number and weaker binding sites for HRM. Conceivable, in the case of C_{18} stationary phase the apolar (aryl rings) part of solvated resorcinarene molecule is connected with the octadecyl chains by –C–H…π bonds and the polar (hydroxyl groups) part of molecule binds to the methanol layer adsorbed on the surface by hydrogen bonds. The C_8 alkyl groups contribute barely to the interaction by –C–H…π bonds therefore a large number of HRM molecules are able to bind to the adsorbed layer of the surface. According to the entropic contribution of case HRM, it can be seen that the affinity of HRM to the stationary phase is more favorable on the C_8 (ΔS = –76.11 J/K mol) than on the C_{18} (ΔS = –82.34 J/K mol) stationary phase (Table 1).

The behavior of MCM is different because the saturation capacity measured on the octadecyl modified silica was larger than that on the C_8 column (Table 7) which means the C_{18} stationary phase has a larger number of stronger binding sites taking into account the K isotherm parameter, too. The entropy changes of MCM on the C_8 (ΔS = –40.69 J/K mol) and C_{18} (ΔS = –46.40 J/K mol) stationary phases do not differ (Table 4). On the C_{18} column, the apolar interactions (-C–H…π bonds) are predominant and presumably the less solvated rigid MCM molecules can penetrate the bulk octadecyl chains resulting in the extremely strong affinity to this stationary phase

5.2.2 Error analysis of estimation of the inverse method

As we discussed, the injected concentrations of solution of HRM and MCM were 0.6 and 3 g/L, respectively. The accuracy of the determination of the isotherm parameters is more reliable when the sample concentration is higher and column overload is more apparent. To understand the bias of isotherm determination caused by the limited analyte solubility, we validated our results. First, random numbers with normal distribution are generated, with the help of Box–Muller method [123]. This method is given as:

$$y_i = \cos 2\pi x_2 \sqrt{-2 \ln x_1} \qquad \text{Eq. 25}$$

where y_i is the generated random number, x_1 and x_2 are two uniform random numbers taken from the [0, 1] interval. The theoretical expected value of generated random numbers is zero and the standard deviation is one.

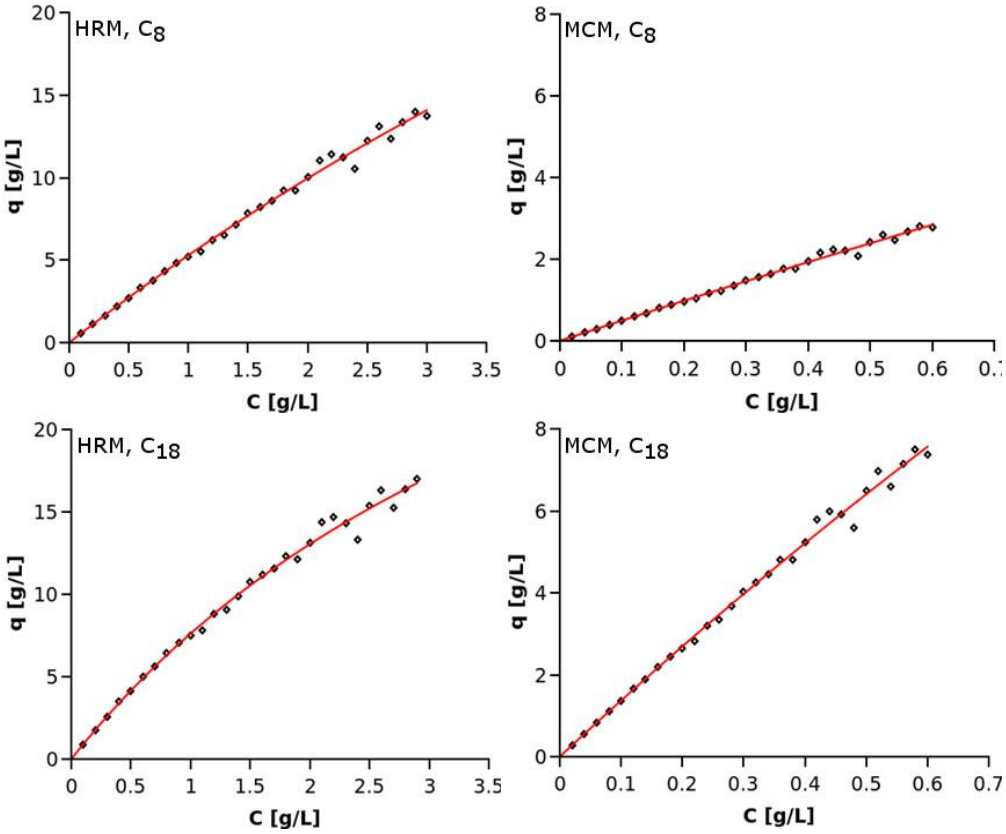

Figure 12. Dots represent the determined Langmuir isotherm data points with the random error (n=0.05); the solid lines represent the fitted Langmuir isotherm model. The analytes and Hypersil BDS columns are (A) HRM, C_8; (B) MCM, C_8; (C) HRM, C_{18} and (D) MCM, C_{18}.

The generated random numbers were applied to calculate noisy isotherm values mimicking experimental errors (χ_i) as we can see in Eq. 37:

$$\chi_i = \left(\frac{nq_i}{q_{\max}} \right) y_i q_i \qquad \text{Eq. 26}$$

where q_{max} is the maximum concentration of analytes on the stationary phases, n is a multiplier and it is equal with 0.01, 0.02 and 0.05, respectively.

Thus, 1, 2 and 5% heteroscedastic random error was considered, respectively. The noise values were added to the series of previously determined q_i data points. In Figure 12, we can see the modified adsorption data points and the fitted Langmuir isotherms. Table 7 shows numerically the influences of the random error on the Langmuir isotherm parameters (determined by IM).

We can conclude that in case of MCM – where the isotherm curvature is barely visible due to the limited analyte solubility – the bias of the isotherm parameters can be 20–30% when 5% random error is added. In case of HRM, the solubility is better and we can achieve larger column overload. Therefore, at 5% random error, the bias of the isotherm parameters is about 5–6%. Even if the estimated isotherm parameters are not entirely accurate, one can get a good estimation regarding the nonlinear behavior of the studied analytes on the various columns.

5.2.3 Comparing the equilibrium constants obtained from the van't Hoff equation and adsorption isotherm

It is interesting to relate the results of linear and nonlinear chromatography. The thermodynamic equilibrium constant (Henry constant) derived from the van't Hoff equation (Eq. 11-12) can be compared with the one calculated from the adsorption isotherm parameters:

$$K = q_s b = e^{\frac{-\Delta G^\circ}{RT}} \qquad \text{Eq. 27}$$

For the examination, we chose the HRM and MCM as analytes and BDS Hypersil C_8 and C_{18} columns as stationary phases.

Table 4. Retention factors and thermodynamic parameters of MCM in methanol-water 88 : 12 (v/v%) mobile phase (T ΔS values are calculated for 303 K).

MeOH/H_2O 88:12 v/v%	BDS C_8	BDS C_{18}
k average	2.10	7.03
ΔS (J K^{-1} mol^{-1})	-40.69	-46.40
ΔH (kJ mol^{-1})	-16.22	-20.50
TΔS (kJ mol^{-1})	-12.33	-14.07
ΔG (kJ mol^{-1})	-3.89	-6.44

The composition of the methanol-containing mobile phase was identical to the solvent of the sample. In each chromatogram, only the main peak was considered. To obtain the adequate adsorption isotherms, the HRM was dissolved in methanol-water 72:28 (v/v%) but the overloaded peak of MCM seemed to be free from minor components only in methanol-water 88:12 (v/v%).

Consequently, we supplemented the thermodynamic measurements of the temperature dependence of the retention factor of MCM with those taken in methanol-water 88:12 (v/v%) mobile phase (Table 4). The values of the Henry constant obtained *via* the two different methods showed a good agreement – the difference between them was within 7% in all cases (Table 5).

Table 5. Comparison of Henry constant (K) of HRM and MCM determined by linear van't Hoff equation and Langmuir model on Hypersil BDS C_8 and C_{18} columns (T = 298.15 K).

Analyte	Eluent	Column	Method	Henry constant
HRM	MeOH/H_2O 72:28 v/v%	BDS C_8	van't Hoff	5.837
			Langmuir	5.619
		BDS C_{18}	van't Hoff	9.357
			Langmuir	9.23
MCM	MeOH/H_2O 88:12 v/v%	BDS C_8	van't Hoff	5.211
			Langmuir	4.96
		BDS C_{18}	van't Hoff	14.74
			Langmuir	13.83

To obtain comparable data for HRM and MCM, we calculated the Henry constant of MCM in methanol-water 72:28 (v/v%) according to Eq. 38 applying

the van't Hoff law. We found that the Henry constant of MCM on the C_8 column (K = 86.3) was 15 times larger and on the C_{18} column (K = 265.4) it was approximately 28 times larger than that of HRM. The results confirm that the affinity of MCM cavitand to the alkyl bonded stationary phase, especially to the octadecyl alkyl chains is fairly high. It is noteworthy that in methanol-water 72:28 (v/v%) a huge increase was observed in comparison with the Henry constants of MCM in methanol-water 88:12 (v/v%) measured on both stationary phases. Hence, we investigated the relationship between the logarithm of Henry constant (according to Eq. 14) and the volumetric composition of organic modifier in both mobile phases and the plots showed linear correlations (Figure 13).

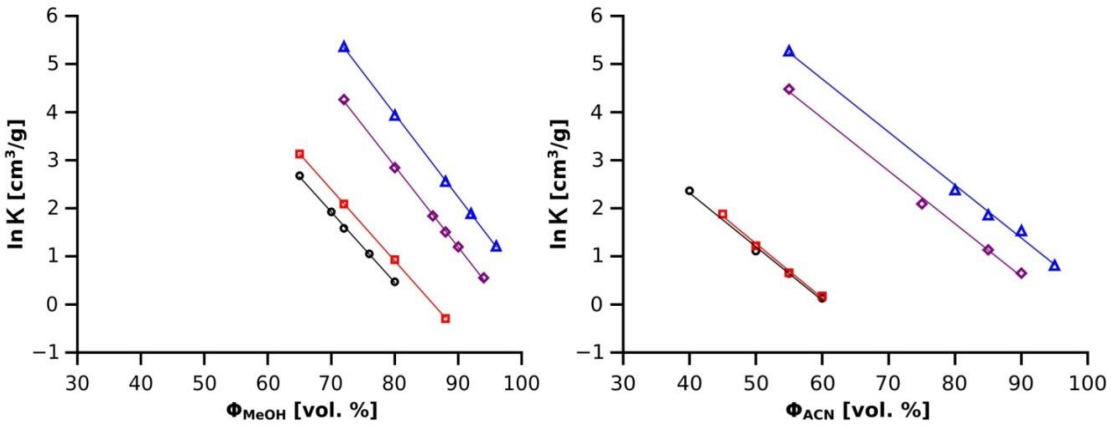

Figure 13. Logarithm of Henry constant (ln K) as a function of volumetric fraction of methanol and acetonitrile, at 303 K. (○) HRM, C_8; (□) HRM, C_{18}; (◊) MCM, C_8 and (Δ) MCM, C_{18}.

The logarithm of Henry constant decreased with increasing volumetric fraction of organic modifier of mobile phases, in a lesser amount in acetonitrile - water than in methanol - water system, according to the slopes of the plots (see Table 6). The ratio of methanol and water had a strong effect on the values of the Henry constant for MCM on both stationary phases. The higher concentration of methanol in the mobile phase caused the drastic decrease of the value of Henry constant because the affinity of the apolar MCM to the mobile phase increases significantly if the methanol content increases.

Table 6. Slope , intercept and correlation coefficient (R^2) of the logarithm Henry constant of HRM and MCM versus the volumetric fraction of methanol (Φ_{MeOH}) and acetonitrile (Φ_{ACN}) as organic modifiers on Hypersil BDS C_8 and C_{18} columns (T = 303 K).

		Φ_{MeOH} [vol. %]			Φ_{ACN} [vol. %]		
		slope	intercept	R^2	slope	intercept	R^2
HRM	BDS C_8	-0.147	12.180	0.999	-0.112	6.788	0.995
	BDS C_{18}	-0.148	12.780	0.999	-0.113	6.939	0.995
MCM	BDS C_8	-0.169	16.360	0.999	-0.110	10.460	0.997
	BDS C_{18}	-0.173	17.780	0.999	-0.110	11.300	0.997

5.3 Temperature dependence of the water adsorption from aqueous acetonitrile on silica-based stationary phases in hydrophilic interaction liquid chromatography

It is generally agreed that in HILIC the retention mechanism involves mostly partitioning between the largely organic mobile phase and a water-rich mobile phase on the surface of stationary phase [95, 108]. However, some authors suppose that this effect is less important with the hydrosilated type silica stationary phases in comparison to the ordinary silica gel [124-126]. In this chapter the water uptake from acetonitrile-rich aqueous–organic mobile phases by frontal analysis using coulometric Karl-Fischer titration is presented. Figure 14 shows examples of the water breakthrough curves for mobile phases with different concentrations of water in acetonitrile, ranging from 0.5 to 15 v/v% in the 40– 100 °C temperature range.

All the breakthrough curves on the columns (A – Cogent Silica C column; B – Cogent Phenyl hydride column) studied have similar profiles with typical sigmoidal shape, in which the inflection point corresponds to the breakthrough volume, which decreases with increasing concentration of water in the acetonitrile–water mobile phases.

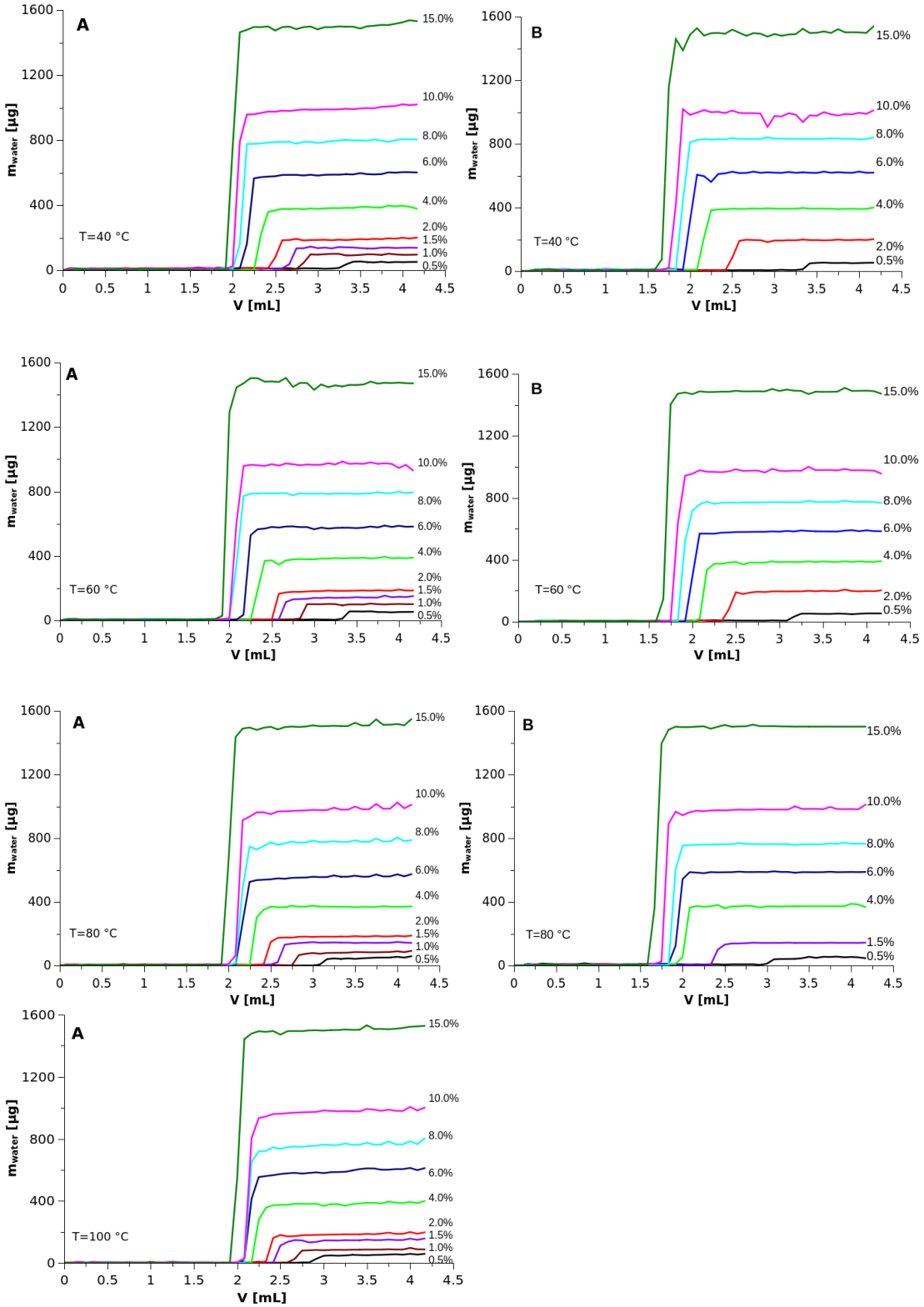

Figure 14. Frontal analysis (breakthrough) plots of water on Cogent Silica C (A) and Cogent Phenyl hydride (B) at different temperatures. Flow rate: 1 mL/min; V– volume of mobile phase; m_{water} –absolute weight of water in 10 µL fraction of mobile phase 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 15.0, (v/v%), water in acetonitrile.

The correct determination of the breakthrough volume is hardly possible if the value of the inflection point of breakthrough curve is too close to the hold-up volume of the column. In case of the Phenyl hydride column if the concentrations of water higher than 90:10 ACN:H_2O (v/v%), than the inflection point of breakthrough curve is too close to the hold-up volume of the column, therefore the correct determination of the breakthrough volume is hardly possible.

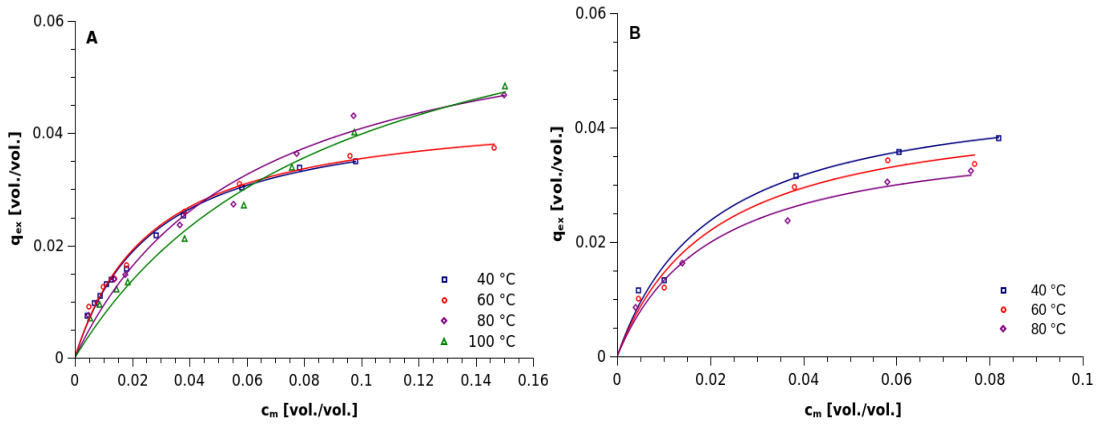

Figure 15. The Langmuir isotherms of water on the hydrosilated silica stationary phases, Cogent Silica C (A) and Cogent Phenyl hydride (B). q_{ex} – column fraction of excess water in the pores of the stationary phase; c_m – volume fraction of water in the mobile phase in equilibrium with the stationary phase.

The concentration of water in the stationary phase should be related to the volume of the stationary phase. However, as there is no fixed boundary between the adsorbed water-rich layer and the bulk mobile phase, we adopted the convention, according to which the amount of water in the stationary phase is expressed as the volume fraction of excess adsorbed water contained within the pores [106].

Figure 15 shows the experimental isotherm profiles which correspond to the classical Langmuir adsorption model, described above at Eq. 16. In this case the isotherm constant *K* is the Henry constant for water adsorption in the pores of the stationary phase at very low concentration of water in the mobile phase (*C*); whereas constant *b* is the equilibrium constant for water adsorption and q_s is the column saturation capacity. The Langmuir isotherm constants were determined by nonlinear regression according to Eq. 16 and are listed in Table

8 together with the adsorbed volume of water (against the water concentration in the bulk mobile phase) retained in the pores of the stationary phase under the saturation conditions (Eq. 39) and the equivalent number of adsorbed monomolecular water layers inside the pore volume at full saturation capacity (Eq. 40)

$$V_{ex} = q_S V_i \qquad \text{Eq. 289}$$

where V_{ex} is the adsorbed volume of water retained in the pores of the stationary phase under the saturation conditions; V_i is the pore volume inside the column.

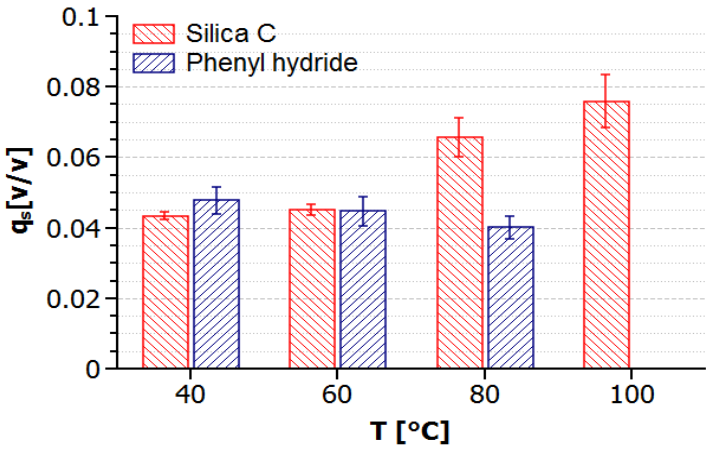

Figure 16. The excess adsorbed water saturation capacity of tested columns at different temperatures

It is interesting to note that in Figure 15 (A) the isotherms obtained at 40 or 60 °C intersect those obtained at 80 or 100 °C. There is no straightforward explanation for that, but the lower saturation capacities obtained at 40 or 60 °C combined with a strong temperature dependence of the equilibrium constant *b*, affects the initial slope of the isotherms, thus intersection occurs. The saturation capacities of the two hydrosilated silica stationary phases at different temperatures are compared in Figure 16, showing that the saturation capacities decrease with increasing of temperatures on Cogent Phenyl hydride but in case of Cogent Silica C they increase with increasing of temperatures. The Silica C column at 40 and 60 °C has approximately 40% lower saturation capacities for water than at the Silica C column at 100 °C and 30% lower than the Silica C

column at 80 °C. On the Phenyl Hydride column saturation capacities slightly decreased with decreasing the temperature. The Henry constants of water (*K*) decrease with increasing temperature, on both investigated columns (Table 8).

Table 8. Langmuir constants *K* and *b*, coefficient of determination R^2, q_s – excess saturation capacity of water (with relative standard deviations), V_{ex} – excess volume of water in the pores, N_w – the equivalent number of adsorbed monomolecular water layers inside the pore volume at full saturation capacity

Columns	T (°C)	K	b [(v/v)$^{-1}$]	q_s (v/v)	R^2	V_{ex} (µL)	N_w
Silica C	40	1.71	39.35	0.043	0.996	37.72	0.392
	60	1.66	36.7	0.045	0.993	39.22	0.408
	80	1.08	16.45	0.066	0.982	57.06	0.593
	100	0.84	11.08	0.076	0.986	65.85	0.684
Phenyl Hydride	40	2.36	49.38	0.048	0.988	32.93	0.283
	60	2.16	48.44	0.045	0.986	30.82	0.265
	80	1.97	49.15	0.040	0.989	27.70	0.238

The Silica C column at 40 and 60 °C has approximately 40% lower saturation capacities for water than at the Silica C column at 100 °C and 30% lower than the Silica C column at 80 °C. On the Phenyl Hydride column saturation capacities slightly decreased with increasing the temperature. The Henry constants of water (*K*) decrease with increasing temperature, on both investigated column (Table 8).

From the column saturation capacity data, the average number of monomolecular water layers per the adsorbent surface inside the pores at full saturation capacity, can be estimated as [106]:

$$N_w = \frac{V_{ex} N_{AV} A_{H_2O} \rho_{H_2O}}{A_s \rho_s V_C (1-\varepsilon_T) M_w{}^{H_2O}}, \qquad \text{Eq. 29}$$

where N_{AV} is the Avogadro constant (6.023×10^{23} mol^{-1}), A_{H_2O}= 18.9×10^{-16} cm^2 is the area occupied by a molecule of water adsorbed on the flat adsorbent surface, calculated from the van der Waals atom radii, ρ_{H_2O} is the density of water (1 g/cm^3), A_s is the specific surface area of silica adsorbents according to the manufacturer's data, ρ_s=2.3 g/cm^3 is the density of silica and $M_w^{H_2O}$ = 18.015 g/mol is the molar mass of water. N_W does not represent the real number of water layers adsorbed on a surface of stationary phase, but it just enables a comparison of uptake of excess water. The data in Table 8 also allow for comparison of values of volume of excess water (V_{ex}) and the equivalent numbers of the adsorbed monomolecular water layers (N_w) on every column and at each temperatures.

On Silica C at full column saturation, i.e. when the concentration of the adsorbed water equals the saturation capacity q_s, the excess adsorbed water fills 4.34% of the pore volume at 40 °C, 4.51% at 60 °C, 6.56% at 80 °C and 7.57% at 100 °C. In case of the Phenyl hydride stationary phase, excess adsorbed water takes 4.77%, 4.47% and 4.02% at 40, 60 and 80 °C, respectively. The amount of water adsorbed in the pores at saturation conditions corresponds to 0.39, 0.41, 0.59 and 0.68 monomolecular water layer for Silica C column at 40, 60, 80 and 100 °C, respectively and furthermore 0.28, 0.27 and 0.24 monomolecular water layer for Phenyl hydride column at 40, 60 and 80 °C, respectively.

Pesek et al. observed that the effects observed for unmodified and modified hydrosilated silica particles can be probably explained by the effects of electrical interfacial double layer of the adsorbent, (characterized by its zeta potential) rather than by the remaining small amount of residual silanol groups [126]. The negative zeta potential on hydrophobic surfaces is supposed to originate in adsorption of hydroxide ions from water auto-dissociation. The negative surface zeta potential was shown to be more negative for more hydrophobic surfaces and is inversely correlated with the amount of excess adsorbed water molecules.

The lower affinity of water at increasing temperatures, as demonstrated by decreasing Henry constant, in Table 8, suggests that the polarity of the Silica C

surface decreases at higher temperatures and consequently the zeta potential decreases, while the saturation capacity and the adsorbed water amount increases.

The presence of phenyl bonded groups increases the polarity of the pore surface in phenyl silica in comparison to unmodified silica C, which may explain higher Henry constant *K* for this column in Table 8. However, the aromatic surface is less hydrophobic than unmodified hydrosilated silica and the effects of temperature on water adsorption are less apparent (Table 8).

6 Conclusion

The retention mechanism of resorcinarenes and cavitands were studied by retention data obtained at different temperatures, which allows the evaluation of the changes in enthalpy, entropy and the Gibbs free energy. Based on the results, it is clear that the transfer of the resorcinarenes and cavitands from the mobile phase to the stationary phase is enthalpy controlled process because the enthalpic contribution for all analytes was found more significant than the entropic contribution of those. It is worth to mention that in acetonitrile-water system the entropic contribution changed in wider range ($T\Delta S$ = –0.5 to –9.7 kJ/mol) than the enthalpic contribution (ΔH = –6.3 to –15.0 kJ/mol), so the difference of the entropy changes plays important role in the different retention behavior of cyclic oligomers.

The binding of the HRM to the stationary phases is the weakest among the investigated cyclic tetramers. In acetonitrile-water system the HRM molecule is the most polar and it can easily create hydrogen bond with water molecules contained in the mobile phase and it is able to accommodate water molecules inside the cavity, thus its affinity to the mobile phase is the strongest. By contrast, if the stationary phase and the solute more apolar, the binding between them is stronger. So the enthalpy change of the MCM on the BDS Hypersil columns has the most negative value, probably due to the strongest formation of –C–H…π bonds among the analytes containing aryl rings. The structural difference between the BDS Hypersil and XTerra stationary phases reflects that the enthalpy and entropy changes of resorcinarenes are almost twice larger on the XTerra RP_8 column than on the BDSHypersil C_8 column and it is 1.3 times as larger on the XTerra RP_{18} column than on the BDS Hypersil C_{18} column in absolute value in acetonitrile-water system. Presumably, one of the reasons for more negative enthalpy change on XTerra columns is that the solvated polar resorcinarene molecules bearing hydroxyl groups are able to create H-bonds with the polar embedded carbamate groups of XTerra stationary phase, but that is not possible in case of BDS Hypersil column containing octadecyl chains. Furthermore, the changes of the entropy are

smaller on the BDSHypersil columns than on the XTerra columns, due to the desolvation of resorcinarene molecules by which they are able to bind to the apolar surface. The combined effect of enthalpy and entropy changes results in more negative Gibbs free energy changes of resorcinarenes on the XTerra columns than on the BDS Hypersil columns. The enthalpy changes and the entropy changes of cavitands are slightly more negative on the BDS columns than on the XTerra columns. The difference of the entropy changes of cavitands on the two types of stationary phases is not significant, because the solvation capability and the freedom of the molecular shape change of the rigid cavitand molecules are slight. As the transfer of the solute from the mobile phase to the stationary phase is enthalpy controlled, the Gibbs free energy changes of cavitands are more negative on the BDS Hypersil columns than on the XTerra columns, therefore the BDS Hypersil stationary phase is more retentive for cavitands.

The huge difference between the retention of resorcinarenes and cavitands is due to the increase of the entropic contribution of cavitands, which causes the more negative values of Gibbs free energy changes on different stationary phases although the enthalpy changes are comparable. The increase of entropic contribution is the reason of the large retention of methylated derivatives, particularly in the case of methyl cavitand, for which the entropy changes (ΔS) are one magnitude smaller than that of the demethylated derivatives on every column in acetonitrile-water system.

The retention behavior of large molecules such as resorcinarenes and cavitands were investigated by linear and nonlinear chromatography too. Comparing the results obtained by analytical chromatography in methanol and acetonitrilecontaining mobile phases, the retention factors of resorcinarenes were significantly larger, while the retention factors of cavitands were smaller in methanol-water than in acetonitrile-water eluent.

Applying methanol modifier in the mobile phase, the enthalpy changes of the analytes became more favorable but the entropic contributions became more unfavorable compared to that in acetonitrile - water. In methanol - water mobile phase, the hydrogen bonds between the solvated analytes and methanol

molecules adsorbed on the surface dominated, therefore every stationary phase was more retentive for resorcinarenes containing hydroxyl groups than the acetonitrile containing mobile phase. In acetonitrile - water eluent the increase of the entropic contribution had a big role to the retention because of the thick adsorbed acetonitrile layer, which was significant in the case of apolar cavitands. Therefore, their retention factors were smaller in methanol-water mobile phase on every column. The effect of polar embedded groups of the XTerra column could manifest in the acetonitrile-water eluent. There was no difference between the enthalpy changes of resorcinarenes on BDS and XTerra columns in methanol-containing mobile phase.

In reversed-phase chromatography pressure generally increases retention. The influence of the change of molar volume on the retention of resorcinarene-based cavitands on alkylsilyl and polar-embedded C_8 and C_{18} reversed phases in aqueous methanol mobile phase was explored. The retention factors of the more polar resorcinarene (HRM) and the apolar cavitand (MCM) increased with the increase of the average column pressure (1 - 400 bar) on each stationary phase. The molar volume changes of HRM calculated from the slope of the plot of ln *k* versus *p.* The values were between -9 -19 mL/mol for HRM and these values for MCM were between -5 -12 mL/mol. The larger molar volume changes of resorcinarene are mostly due to the larger loss of its solvation layer and furthermore its conformational changes when it interacts with the apolar ligands of the stationary phase. According to a raw estimation, the volume change of a single molecule seems to be only a few percent of the total volume of the solvated HRM or MCM molecule.

Both types of the analytes had twice larger molar volume change on the Hypersil BDS than on the XTerra C_8 or C_{18} columns. The reason for that is that the polar-embedded phase can take up more solvent and is more ordered via hydrogen bonds than its alkyl counterpart. Thus, the analyte transport from the mobile to the stationary phase results in a lesser loss of the solvation layer of the molecules. The alkyl chain length of the ligands has no influence on the molar volumes of the analytes.

To better understand the retention mechanism, the adsorption properties of the analytes on BDS Hypersil C_8 and C_{18} stationary phases were studied by the nonlinear chromatography. The inverse method was chosen for the determination of adsorption isotherms as the solubility of the examined resorcinarene (HRM), especially the cavitand (MCM) was very poor. The Langmuir isotherm represented well the experimental data, which corresponds to a homogeneous adsorbent.

The calculated adsorption – desorption equilibrium constant of HRM and MCM was three times and twice larger on the C_{18} than on the C_8 column, respectively. The *b* isotherm parameter is connected with the adsorption energy of the analytes. According to expectations, the results refer to larger adsorption energy between the analytes and the octadecyl chains than the octyl chains of the stationary phase. The saturation capacity of HRM was twice larger but this parameter of MCM was smaller on the C_8 than on the C_{18} column. The solvated HRM molecules containing polar and apolar groups can create two types of interaction (hydrogen and –C– H…π bonds) with the stationary phase and they can create a smaller number of stronger binding sites on the octadecyl bonded silica and a larger number of weaker binding sites on the octyl bonded column. The less solvated rigid MCM molecules can penetrate into the bulk octadecyl chains resulting in the extremely strong affinity to this stationary phase. This means that there is a large number of high-energy binding sites on the C_{18} column, as also reflected by the value of calculated Henry constant. We obtained for MCM approximately 28 times larger Henry constant than for HRM on the C_{18} column. On the C_8 column, the value of K of MCM was 15 times larger than that of HRM. The Henry constants of HRM and MCM calculated by linear and nonlinear chromatography showed a good agreement.

As the sample size was limited by the solubility of the analytes for the determination of an adsorption isotherm, I made an error analysis of the isotherm parameters with generation 1, 2 and 5% heteroscedastic random error. The bias of the isotherm parameters of MCM can be 20 – 30% when 5% random error is added and in case of HRM this bias rises only about 5 – 6%. The estimations obtained with the inverse method are valid over a broader

concentration range, so we can get a good estimation of the nonlinear behavior of the analytes.

The temperature dependence of hydrophobicity was studied on Silica C and Phenil hydride (two different types of hydrosilated silica) stationary phases by frontal analysis method with the Karl Fischer determination of water in the column effluent. The results showed relatively low preferential adsorption of water from aqueous acetonitrile, probably due to surface zeta potential of the stationary phase.

The Silica C stationary phase shows the equivalent numbers of the adsorbed monomolecular water layers, N_w, equivalent to 0.39, 0.41, 0.59 and 0.68 at 40, 60, 80 °C and 100 °C, respectively. On the Phenyl Hydride column, the number of adsorbed layers of water molecules was equal to 0.28 at 40 °C, 0.27 at 60 °C and 0.24 at 80 °C. The adsorption isotherms of water can be described by Langmuir model, like in non-aqueous normal-phase LC. At full column saturation, the adsorbed water fills 4.02 – 7.57% pore volume over the studied temperature range, which approximately corresponds to the equivalent of 0.24 – 0.68 water layer coverage of the adsorbent surface. On the Silica C column, the adsorbed excess water monomolecular water equivalents, N_w increase with increasing temperatures whereas on the Phenyl Hydride column the saturation capacity and the N_w are not significantly affected by the temperature.

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8 Thesis points

1. Based on the determined thermodynamics parameters, it is clear that the transfer of the resorcinarenes and cavitands from the mobile phase to the stationary phase is enthalpy controlled process because the enthalpic contribution for all analytes was found more significant than the entropic contribution of those.
2. In acetonitrile - water system the huge difference between the retention of resorcinarenes and cavitands is due to the increase of the entropic contribution of cavitands, which causes the more negative values of Gibbs free energy changes on different stationary phases although the enthalpy changes are comparable. The increase of entropic contribution is the reason of the large retention of methylated derivatives, particularly in the case of MCM, for which the entropy changes (ΔS) are one magnitude smaller than that of the demethylated derivatives on every column.
3. Applying methanol modifier in the mobile phase, the enthalpy changes of the analytes became more favorable but the entropic contributions became more unfavorable compared to that in acetonitrile-water.
4. The calculated adsorption–desorption equilibrium constant of HRM and MCM was three times and twice larger on the C_{18} than on the C_8 column, respectively.
5. It is found that the molar volume changes for both the apolar and more polar analytes were twice larger on the Hypersil BDS than on the XTerra columns and they were independent of the length of the alkyl chains of the stationary phases.
6. In case of the temperature dependence of hydrophobicity experiment, the adsorbed monomolecular water layer was calculated. It enables a comparison of the uptake of excess water.

9 List of Symbols

G^S and G^M	Gibbs free energy. Superscripts *S* and *M* denote the stationary and the mobile phases, respectively
μ_i	the chemical potential of *i*
c_i	concentration of *i* analyte
R	ideal gas constant
T	temperature
V_S	volume of stationary phase
V_M	volume of mobile phase
K	the Henry's constant or simply the ratio of the analyte concentrations in the stationary and the mobile phases at equilibrium
ΔH	enthalpy change of the reversible binding of the analyte
ΔS	entropy change of the reversible binding of the analyte
ΔG	Gibbs free energy change of the reversible binding of the analyte
V_S/V_M	the phase ratio
ΔE	internal energy of the reversible binding of the analyte
ΔV_m	the change of molar volume of the solute
p	pressure
C	concentration of the analyte in the mobile phases
q	the concentration of the analyte in the stationary phases
q_s	saturation capacity

b	adsorption–desorption equilibrium constant of the solute from the solution onto the bare surface of the adsorbent
b_L	the equilibrium constants of the solute from the solution into a layer of adsorbate molecules
v	heterogeneity parameter
V_B	the retention volume of the self-sharpening shock (breakthrough volume)
V_M	the system holdup volume
V_{ads}	the adsorbent material filling the column
Z	the distance along the column
t	the time
u	the mobile phase linear velocity
D_a	the apparent axial dispersion coefficient
H	the height equivalent to a theoretical plate (HETP) for the component considered
t_M	is the void time
L	the length of the column
F_v	flow rate
V_0	interstitial volume in between the particles
ε_T	total column porosity
V_C	volume of empty tube
V_i	pore volume inside the column
m	amount of the injected sample
A	absorbance

q_{ex}	*the excess concentration of water adsorbed on the stationary phase*
y_i	generated random number
x_1 and x_2	two uniform random numbers taken from the [0, 1] interval
χ_i	experimental errors
q_{max}	maximum concentration of analytes on the stationary phases
n	a multiplier and it is equal with 0.01, 0.02 and 0.05, respectively
V_{ex}	the adsorbed volume of water retained in the pores of the stationary phase under the saturation conditions
N_{AV}	Avogadro constant (6.023×10^{23} mol^{-1})
A_{H_2O}	18.9×10^{-16} cm^2 is the area occupied by a molecule of water adsorbed on the flat adsorbent surface, calculated from the van der Waals atom radii
ρ_{H_2O}	density of water (1 g/cm^3)
A_s	specific surface area of silica adsorbents according to the manufacturer's data
ρ_s	density of silica (2.3 g/cm^3)
$M_W^{H_2O}$	the molar mass of water
N_W	number of water layers adsorbed on a surface of stationary phase (note: it does not represent the real number of water layers adsorbed on a surface of stationary phase, but it just enables a comparison of uptake of excess water)

10 Glossary

ACN	acetonitrile
BET	Brunauer-Emmett-Teller procedure
HETP	height equivalent to a theoretical plate
HILIC	Hydrophilic interaction chromatography
HPLC	High Performance Liquid Chromatography
IUPAC	International Union of Pure and Applied Chemistry
FA	Frontal analysis
IM	Inverse method
MeOH	methanol
THF	tetrahydrofuran
UHPLC	Ultra-High Performance Liquid Chromatography

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12 Publications

12.1 Publications related to the thesis

1. I. Prauda, **E. Bartó** and A. Felinger, "Influence of pressure on the retention of resorcinarene-based cavitands", *Journal of Chromatography A,* vol. 1535, pp. 123-128, 2018. Impact Factor: 3.981

2. **E. Bartó**, A. Felinger and P. Jandera, "Investigation of the temperature dependence of water adsorption on silica-based stationary phases in hydrophilic interaction liquid chromatography", *Journal of Chromatography A*, vol. 1489, pp. 143-148, 2017. Impact Factor: 3.981

3. **E. Bartó**, I. Prauda, F. Kilár, I. Kiss and A. Felinger, "Investigation of retention mechanism of resorcinarene based cavitands by linear and nonlinear chromatography", *Journal of Chromatography A*, vol. 1456, pp. 152-161, 2016. Impact Factor: 3.926

4. **E. Bartó**, I. Prauda, F. Kilár, I. Kiss and A. Felinger, "Retention behavior of resorcinarene-based cavitands on C_8 and C_{18} stationary phases", *Journal of Separation Science*, vol. 38, no. 17, pp. 2975-2982, 2015. Impact Factor: 2.741

5. **E. Bartó**, A. Felinger, F. Kilár, I. Prauda and I. Kiss, "The Temperature Dependence of the Retention Factor of Cavitands on C_{18} and C_8 Reversed Stationary Phases in HPLC", *Műszaki Szemle*, vol. 63, pp. 17-22, 2013.

12.2 Posters and presentations related to this thesis

1. **E. Bartó**, I. Prauda, F. Kilár, I. Kiss and A. Felinger; Kavitandok nemlineáris izotermájának meghatározása különböző fordított fázisú állófázisokon az inverz módszer segítségével; 20th International conference on chemistry, November 6-9, Kolozsvár (Cluj-Napoca, Romania), 2014

2. **E. Bartó**, I. Prauda, F. Kilár, I. Kiss and A. Felinger; Kavitandok retenciós tényezőjének hőmérséklet függése különböző fordított fázisú állófázisokon; 19th International conference on chemistry, November 21-24, Nagybánya(Baia-Mare, Romania), 2013

3. **E. Bartó**, I. Prauda, F. Kilár, I. Kiss, V. Sándor and A. Felinger; Study of resorcin[4]arene derivatives in Reversed-Phase High-Performance Liquid Chromatography coupled Mass Spectrometry; 10th János Szentágothai Transdisciplinary Conference and Student Competition, November 4-5, Pécs (Hungary) 2013

4. **E. Bartó**, A. Felinger and P. Jandera; Investigation of Temperature Dependence of Adsorption of Water from Aqueous Acetonitrile on Silica Based Stationary Phases; 10th Balaton Symposium on High-Performance Separation Methods, September 6-8, Siófok (Hungary) 2015

5. **E. Bartó**, I. Prauda, F. Kilár, I. Kiss and A. Felinger; Kavitandok retenciós jelenségének túlterheléses vizsgálata különböző állófázisokon; Elválasztástudományi Vándorgyűlés, October 12-14, Egerszalók (Hungary) 2014

6. **E. Bartó**, I. Prauda, F. Kilár, I. Kiss and A. Felinger; Investigation of the retention phenomena of cavitands by column overload; 30th International Symposium on Chromatography, September 14-18, Salzburg (Austria) 2014

7. **E. Bartó**, I. Prauda, F. Kilár, I. Kiss and A. Felinger; Temperature dependence of the retention factors of resorcinarene based cavitands on C_8 and C_{18} reversed stationary phases; 30th international symposium on microscale bioseparations, April 27 – May 1, Pécs (Hungary) 2014

8. **E. Bartó**, I. Prauda, F. Kilár, I. Kiss, V. Sándor and A. Felinger; Analisys of resorcin[4]arene-based cavitand on different stationary phases; 9th Balaton Symposium on High-Performance Separation Methods, September 4-6, Siófok (Hungary) 2013

9. **E. Bartó**, I. Prauda, F. Kilár, I. Kiss, V. Sándor and A. Felinger; Analysis of cavitand derivatives with High Performance Liquid Chromatography – Mass Spectrometry; 39th International Symposium on High Performance Liquid Phase Separations and Related Techniques, June 16-20, Amsterdam (Netherland) 2013

10. **E. Bartó**, I. Prauda, F. Kilár, I. Kiss and A. Felinger; Application of Cavitand Derivatives on High Performance Liquid Chromatography; 10th International Interdisciplinary Meeting on Bioanalysis, April 25-27, Pécs (Hungary) 2013