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FINAL REPORT

## INTRODUCTION

The central part of our investigation was the study of the interaction of analyte molecules with stationary phases. We have employed several tools to characterize the solute or solvent adsorption on HPLC stationary phases, and the mass transfer properties of small and macromolecules in HPLC columns.

## THE PORE SIZE DISTRIBUTION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY STATIONARY PHASES [1,2]

Inverse size exclusion chromatography is an accurate method to investigate the structure of a porous HPLC packing material without destroying the column. The stochastic theory of size exclusion chromatography with pore size distribution was used to calculate the pore sizes and pore size distributions of various commercially available HPLC columns; four of them were packed with superficially porous particles and three were packed with totally porous particles.

The wide pore size distribution model of SEC always fits significantly better to the experimental results than the monopore model. Using the result obtained with the fitting the wide pore size distribution model to determine the porosities of HPLC columns, we can get a realistic view of the structure of stationary phases and chose the most appropriate column to separate a complex sample.

## THE PORE SIZE DISTRIBUTION OF THE FIRST AND THE SECOND GENERATION OF SILICA MONOLITHIC STATIONARY PHASES [3]

The stochastic theory of size-exclusion chromatography with pore size distribution was used to estimate the pore size and the pore size distribution of the 1G Chromolith Performance and the 2G Chromolith High-Resolution monolithic columns. Inverse size-exclusion chromatography was the chosen method because of its accuracy and stationary phase friendly properties.

One can observe a significant difference between the SEC calibration curves on the two investigated columns. The slopes of the curves are entirely different in the hydrodynamic range, i.e. for the molecules excluded from the mesopores. From the hydrodynamic effect, the sizes of the macropores were determined. The macropore sizes were found to be  $r_0 = 1.55$  and  $0.95 \mu\text{m}$  in case of 1G and 2G monoliths, respectively.

The pore structure and morphology of the silica-based monolithic columns have been thoroughly studied with different approaches, such as mercury intrusion porosimetry, scanning electron microscopy and confocal laser scanning microscopy, etc. Our investigation has revealed that simple chromatographic experiments – inverse size-exclusion chromatography in this instance – can also provide substantial and accurate information regarding the pore structure of monolithic columns. The combination of ISEC experiments with a proper theory can be an attractive alternative to characterize the mesopores and macropores of stationary phases with tools that are present in a chromatography laboratory.

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#### POLYDISPERSITY IN SIZE-EXCLUSION CHROMATOGRAPHY [4]

The stochastic theory of size-exclusion chromatography was extended for polydisperse samples i.e. where the size of the molecules is described by a distribution. The results presented verify previous observations and experiences: a significant band-broadening and loss in the selectivity can be observed for polydisperse samples. By integrating polydispersity into the stochastic theory of chromatography we have the proper tools to investigate the effect of polydispersity and that of the pore geometry on the chromatograms and on the moments at the same time. Although the effect of zone broadening caused only by polydispersity could not be evaluated experimentally, its effect is very important and should be understood to optimize the calibration processes and the separations.

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#### MASS-TRANSFER PROPERTIES OF INSULIN ON CORE-SHELL AND FULLY POROUS STATIONARY PHASES [5]

According to the results of the general rate model, there is no significant difference between the different columns in respect of the intraparticle diffusion coefficients. The separate determination of the pore diffusion and the surface diffusion coefficients shows that the latter has a crucial role in the intraparticle movement of the macromolecules. The values of the pore diffusion coefficients are at least one order of magnitude lower than the values of the surface diffusion coefficients. However, significant differences were obtained in the pore diffusion between the fully porous columns and the core-shell packed columns. The explanation is the dissimilarities of the intraparticle obstruction and of the hindrance factors. In this study, we found that the intraparticle diffusion coefficients, and so, the surface diffusion coefficients are dependent on the mobile phase velocities. This is due to the pressure dependence of the retention of insulin sample.

According to the stochastic analysis, the average sojourn times that molecules spend in the stationary phase of the core-shell packed columns are at least the half of those of the fully porous packings. Consequently, the number of the adsorption events of molecules in case of the partially porous columns is nearly double of that in case of the conventional fully porous media.

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#### THE INFLUENCE OF THE DATA ACQUISITION RATE ON THE DIGITIZED CHROMATOGRAM [6]

With the need for high-frequency data acquisition, the influence of the data acquisition rate on the quality of the digitized signal is often discussed and also misinterpreted.

We demonstrated that data acquisition rate has no influence at all on band broadening and hence resolution. What some researchers observe is not the effect of sampling rate, but it is the consequence of undocumented software features. Loss of efficiency or resolution may indeed be observed when a modern detector is set to a slow sampling rate. The loss of efficiency, however, occurs when instrument manufacturers think that a good signal-to-noise ratio is more important than faithful representation of the original signal and they sacrifice peak shape by coupling a digital filtering to data acquisition.

Another important result is that a modern fast detector cannot always be used to emulate the performance of slow detectors, since when a fast detector is run at slow data acquisition rate, the inner clock still digitalizes the analog signal at a fast rate, but the digital manipulation or signal processing step alters the shape of the signal.

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#### COLUMN PERFORMANCE IN SUPERCRITICAL FLUID CHROMATOGRAPHY AND IN LIQUID CHROMATOGRAPHY [7]

We have studied the chromatographic behavior of the homologous series of alkylbenzenes (from octylbenzene to octadecylbenzene) on the same C<sub>18</sub> reversed-phase column in supercritical fluid chromatography (SFC) and

reversed phase liquid chromatography (RPLC) at various experimental conditions, such as different eluent compositions, flow-rates, and mobile phase densities.

The results indicate that in SFC – as the density of the mobile phase is influenced by the flow-rate – there is a broader variation of mass-transfer properties than in liquid chromatography. As expected, the optimum mobile phase velocity is higher in SFC, but there is no real difference in the minimum value of plate height, i.e. in the optimum efficiency.

#### INVESTIGATION OF RETENTION MECHANISM OF RESORCINARENE BASED CAVITANDS BY LINEAR AND NONLINEAR CHROMATOGRAPHY [8,9]

Cavitands are cavity-shaped cyclic oligomers and they can create host–guest interactions with various analytes, therefore they have applications in supramolecular chemistry, nanoscale reactions, chromatographic separations, drug encapsulation and delivery, biochemistry. The investigation of the chromatographic behavior of large molecules, such as resorcinarenes and cavitands is meager up to now. To understand the retention of resorcinarenes and cavitands in liquid chromatography, we studied their retention mechanism by the thermodynamic parameters calculated from the van 't Hoff equation and by generation of an adsorption isotherm, which can describe the adsorption of the solute on the stationary phase surface. We compared the thermodynamics of the retention for cyclic oligomers in acetonitrile–water and methanol–water mobile phases. Furthermore, we determined the equilibrium adsorption isotherm by inverse method, and we made an error analysis of the estimation obtained with the inverse method to ascertain the validity of the obtained isotherm parameters over a broader concentration range.

#### INFLUENCE OF PARTICLE SIZE AND SHELL THICKNESS OF CORE-SHELL PACKING MATERIALS ON OPTIMUM EXPERIMENTAL CONDITIONS IN PREPARATIVE CHROMATOGRAPHY [10]

The applicability of core-shell phases in preparative separations was studied by a modeling approach. The results show that as the size of the core increases, larger particles can be used in separations, resulting in higher applicable flow rates, shorter cycle times. Due to the decreasing volume of porous layer, the loadability of the column dropped significantly. As a result, the productivity and economy of the separation decreases. We showed that if it is possible to optimize the size of stationary phase particles for the given separation task, the use of core-shell phases is not beneficial. The use of core-shell phases proved to be advantageous when the goal is to build preparative column for general purposes (e.g. for purification of different products) in small scale separations.

#### COMPARISON OF THE KINETIC PERFORMANCE OF DIFFERENT COLUMNS FOR FAST LIQUID CHROMATOGRAPHY, EMPHASIZING THE CONTRIBUTIONS OF COLUMN END STRUCTURE [11]

The kinetic performance of chromatographic columns designed for fast liquid chromatography with different column packing materials – including fully porous, core-shell or monolithic packings – with identical column dimensions was tested. Since the tested monolithic column showed systematically better efficiency for early eluting compounds than the packed columns, an additional band broadening effect was suspected for the packed columns. The effects of the presence of the frits and the bed heterogeneity of the columns near the frits were characterized by a column-reversal method. It has been shown that significant differences – even 20–25% difference inefficiency – can exist between the two ends of the packed columns, while the monolithic column shows rather similar performance at either column end.

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## TWO-DIMENSIONAL CORRELATION ANALYSIS OF THE REPRODUCIBILITY OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COLUMNS [12]

Two-dimensional (2D) correlation analysis is a well-established tool in spectroscopy. Despite its versatility in various measurement systems, 2D correlation has not yet become popular in separation science. 2D correlation is seldom used in chromatography; only a few studies can be found on this topic and most of those publications report about gel chromatography.

We introduced a simple method for studying the similarities and dissimilarities between a number of chromatograms. We presented the applicability of the method by two examples, where the repeatability and reproducibility of the analytical and nonlinear measurements in HPLC are evaluated and demonstrated. In order to validate the results of 2D correlation analysis, they are compared to principal component analysis (PCA). We confirmed the equivalence in the interpretation of the results obtained with the two methods of calculation. The results confirm that 2D correlation can be a successful chemometric tool in chromatography.

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## CORRELATION ANALYSIS ON 3D DATA – INTRODUCING THE ALTERATION ANALYSIS [13,14]

Alteration analysis is established to investigate 3D data composed of a series of 2D measurements taken in an order of a perturbation. Alteration analysis is somewhat similar to 2D correlation analysis but has different attributes. It can show otherwise hardly accessible properties of 3D measurement system with only a few straightforward maps. It can separate a variety of changes on these maps. It shows that with a simple mathematical approach, a versatile method can be built. Alteration analysis can work with any kind of data. Consequently, it can be a useful chemometric tool as a part of the evaluation of a large scale of analytical experiments.

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## MULTILAYER ADSORPTION ON FRACTAL SURFACES [15]

Multilayer adsorption is often observed in liquid chromatography. The most frequently employed model for multilayer adsorption is the BET isotherm equation. In this study we introduce an interpretation of multilayer adsorption measured on liquid chromatographic stationary phases based on the fractal theory. The fractal BET isotherm model was successfully used to determine the apparent fractal dimension of the adsorbent surface. The nonlinear fitting of the fractal BET equation gives us the estimation of the adsorption equilibrium constants and the monolayer saturation capacity of the adsorbent as well. In our experiments, aniline and proline were used as test molecules on reversed phase and normal phase columns, respectively. Our results suggest an apparent fractal dimension 2.88–2.99 in the case of reversed phase adsorbents, in the contrast with a bare silica column with a fractal dimension of 2.54.

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## MULTILAYER ADSORPTION IN LIQUID CHROMATOGRAPHY – THE SURFACE HETEROGENEITY BELOW AN ADSORBED MULTILAYER [16]

A numerical method was introduced for the estimation of the surface heterogeneity below an adsorbed multilayer of the analyte. The calculation procedure is based on the raw adsorption isotherm data points obtained by frontal analysis experiments. To permit the mapping of the nature of the analyte–surface interaction, a numerical procedure was used to pre-estimate the adsorbate–adsorbate interactions occurring during the adsorption process. The surface heterogeneity estimation was carried out using the affinity-energy distribution calculations with assuming local BET isotherm. In the local BET isotherm, the pre-estimated adsorbate–adsorbate interaction constant was used, and the surface heterogeneity was described. After the test of the numerical method with benchmark isotherms, the algorithm was tested on several experimental isotherms. The

isotherms were measured using phenol as test molecule on reversed phase adsorbents, with different surface coverage of the octadecyl ligands. The surface of the non-end-capped stationary phases showed detectable heterogeneity, while the surface end-capped phases were found to be homogeneous.

#### TEMPERATURE DEPENDENCE OF WATER ADSORPTION ON SILICA-BASED STATIONARY PHASES IN HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY [17]

The adsorption of water was investigated in aqueous normal-phase liquid chromatography on Cogent Silica C and Cogent Phenyl hydride stationary phases at different temperatures by frontal analysis – using coulometric Karl Fischer titration – to compare the temperature dependence of adsorption of water from aqueous acetonitrile. The Cogent Silica-C and Cogent Phenyl Hydride columns have a silicon hydride surface (silica hydride) with less than 2% free silanol group; therefore, they do not have a strong association with water. The adsorption behavior of water on the mentioned stationary phases was modeled by Langmuir isotherm. The preferentially adsorbed water was expressed in terms of a hypothetical monomolecular water layer equivalent in the inner pores. The uptake of water slightly depends on the temperature. The adsorbed water may fill four to eight percent of the 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. The phenyl hydride stationary phase shows decreased water uptake in comparison to the Silica C stationary phase.

#### INFLUENCE OF PRESSURE ON THE RETENTION OF RESORCINARENE-BASED CAVITANDS [18]

The thermodynamics of the retention mechanism of resorcinarene-based cavitands in RPLC as well as the nature of the binding sites have been studied recently. In the present study, the influence of pressure on the retention of the cyclic tetramers on alkylsilyl and polar-embedded C8 and C18 stationary phases is investigated using aqueous methanol mobile phase. The pressure effect for cavity-shaped molecules has been scarcely studied so far. We observed that the retention factors of the analytes increased with the increase of the average column pressure (1–400 bar) when using restricting capillary tubes. The calculated molar volume changes were negative, between  $-\Delta V_m = 5\text{--}19$  mL/mol on all types of stationary phases. Comparing the different stationary phases, we found that the molar volume changes for both the apolar and more polar analytes were twice larger on the Hypersil BDS (base deactivated silica) than on the XTerra columns and they were independent of the length of the alkyl chains of the stationary phases.

#### REFERENCES

1. Annamária Sepsey, Ivett Bacskay, Attila Felinger: Molecular theory of size exclusion chromatography for wide pore size distributions, *Journal of Chromatography A*, 1331, 52–60, 2014
2. Ivett Bacskay, Annamária Sepsey, Attila Felinger: Determination of the pore size distribution of high-performance liquid chromatography stationary phases via inverse size exclusion chromatography, *Journal of Chromatography A*, 1339, 110–117, 2014
3. Ivett Bacskay, Annamária Sepsey, Attila Felinger: The pore size distribution of the first and the second generation of silica monolithic stationary phases, *Journal of Chromatography A*, 1359, 112–116, 2014
4. Annamária Sepsey, Ivett Bacskay, Attila Felinger: Polydispersity in size-exclusion chromatography: A stochastic approach, *Journal of Chromatography A*, 1365, 156–163, 2014
5. Nándor Lambert, Ibolya Kiss, Attila Felinger: Mass-transfer properties of insulin on core-shell and fully porous stationary phases, *Journal of Chromatography A*, 1366, 84–91, 2014
6. Attila Felinger, Anikó Kilár, Borbála Boros: The myth of data acquisition rate, *Analytica Chimica Acta* 854, 178–182, 2015

7. Nándor Lambert, Attila Felinger: Performance of the same column in supercritical fluid chromatography and in liquid chromatography, *Journal of Chromatography A* 1409, 234-240, 2015
8. Endre Bartó, Ibolya Prauda, Ferenc Kilár, Ibolya Kiss, Attila Felinger: Retention behavior of resorcinarene-based cavitands on C8 and C18 stationary phases, *Journal of Separation Science* 38, 2975-2982, 2015
9. Endre Bartó, Ibolya Prauda, Ferenc Kilár, Ibolya Kiss, Attila Felinger: Investigation of retention mechanism of resorcinarene based cavitands by linear and nonlinear chromatography, *Journal of Chromatography A* 1456, 152–161, 2016, 2016
10. Krisztián Horváth, Attila Felinger: Influence of Particle Size and Shell Thickness of Core-Shell Packing Materials on Optimum Experimental Conditions in Preparative Chromatography, *Journal of Chromatography A* 1407, 100-105, 2015
11. Nándor Lambert, Shota Miyazaki, Masayoshi Ohira, Nobuo Tanaka, Attila Felinger: Comparison of the kinetic performance of different columns for fast liquid chromatography, emphasizing the contributions of column end structure, *Journal of Chromatography A* 1473, 99–108, 2016, 2016
12. József Simon, Attila Felinger: Two-dimensional correlation analysis of the reproducibility of high-performance liquid chromatography columns, *Journal of Chromatography A* 1384, 115-123, 2015
13. József Simon, Attila Felinger: Correlation analysis on 3D data – Introducing the alteration analysis, *Chemometrics and Intelligent Laboratory Systems* 158, 54–60, 2016, 2016
14. József Simon, Attila Felinger: Exploring the changes in a series of measurements – The comparison of the two-dimensional correlation analysis and the alteration analysis, *Chemometrics and Intelligent Laboratory Systems* 168 (2017) 28–37, 2017
15. Péter Vajda, Attila Felinger: Multilayer adsorption on fractal surfaces, *Journal of Chromatography A* 1324, 121–127, 2014
16. Krisztián Horváth, Péter Vajda, Attila Felinger: Multilayer adsorption in liquid chromatography – The surface heterogeneity below an adsorbed multilayer, *Journal of Chromatography A*, 1505 (2017) 50–55, 2017
17. Endre Bartó, Attila Felinger, Pavel Jandera: Investigation of the temperature dependence of water adsorption on silica-based stationary phases in hydrophilic interaction liquid chromatography, *Journal of Chromatography A* 1489 (2017) 143–148, 2017
18. Ibolya Prauda, Endre Bartó, Attila Felinger: Influence of pressure on the retention of resorcinarene-based cavitands, *Journal of Chromatography A* 1535 (2018) 123–128, 2018

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