RETENTION IN ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY AND IN SUPERCRITICAL FLUID CHROMATOGRAPHY

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FINAL REPORT

INTRODUCTION

The central part of our investigation was the study of retention in the practice of modern chromatography. A good number of phase systems, stationary phases are available for separations. The use of ultraperformance liquid chromatography results in fast separations at pressures over 1000 bars. Supercritical fluid chromatography offers an attractive alternative to liquid chromatography. Due to the low viscosity of supercritical fluids, SFC systems are operated at high mobile phase flow rates resulting in short analysis times.

The better understanding of the mass transfer processes makes it possible that – taking into account the nature of compounds to be separated – one can select the proper separation phase or packing material and can optimize the separation of environmental and biological samples by either UPLC or SFC method.

SFC brings many surprises that are not witnessed in liquid chromatography. A profound investigation is needed to better understand the unexpected features of SFC.

THE EFFECT OF THE FRICTIONAL HEAT ON RETENTION AND EFFICIENCY IN THERMOSTATED OR INSULATED CHROMATOGRAPHIC COLUMNS PACKED WITH SUB-2-μM PARTICLES [1]

The mass-transfer properties of a core-shell packing material with 1.6 µm particle diameter, and that of a fully porous packing material with 1.7 µm particle diameter were investigated and compared. The first absolute and the second central moments of the peaks of the homologous series of alkylbenzenes, over a wide range of mobile phase velocities were measured and used for the calculation of the mass-transfer coefficients. For the evaluation of the band broadening caused by the thermal dissimilarities, the measurements were carried out under thermostated conditions and at near adiabatic insulation of the columns.

Our research has demonstrated from experimental viewpoint that the mobile phase dispersion in columns packed with sub-2-µm packings is significantly affected by the heat loss through their walls. This heat flux originates from frictional heat of the mobile phase against the stationary packed bed and causes crucial efficiency loss at elevated flow rates. By the estimation of the different plate height contributions, the band broadening originating from the effect of the frictional heat was identified. According to our findings, the efficiency loss due to the temperature heterogeneities in the column is in the same range as the loss that originates from the external mass-transfer resistance and axial dispersion contributions altogether. Thermal heterogeneities are present in the same extent in the core-shell type and in the fully porous packing materials. We could not clearly prove that there are differences in the behavior of the core-shell like or fully porous packing materials regarding the frictional heat effects. In case of a core-shell column, abound 45%, whereas for a fully porous packing, 60–65% of the total plate height can be attributed to intraparticle mass-transfer processes. The column insulation intro-duces a 20–25% decrease of the reduced plate height values at high flow rates. There is no correlation between the retention factor and the effect of the frictional heat on the reduced plate height.

RATE CONSTANT DETERMINATION OF INTERCONVERTING ENANTIOMERS BY CHIRAL CHROMATOGRAPHY USING A STOCHASTIC MODEL [2]

It may happen under the conditions employed that enantiomers interconvert to each other. In this case, obviously, the kinetics of the process is to be examined. When enantiomers dynamically interconvert to each other during the separation process, a plateau is observed between the adjacent peaks (so-called Batman peak appears). The peak shape depends on the rate constant of this dynamic reaction. A novel stochastic model was derived which takes both the separation and the interconversion into account at the molecular level – thus the effects of the parameters affecting the separation can be investigated. The novel model was used for the study of quetiapine, a drug molecule that interconverts during the separation to evaluate the rate constant based on the enantiomerization. Various flow rates and temperatures were used, and good agreement was obtained with the rate constant obtained from optical rotation experiments and with the software written by Trapp. The most important result we concluded is the need of mild conditions during the separation to ascertain the rate constant the most accurately (low flow rates and temperatures where the enantiomerization process is limited to a few interconversions). The comparison of the rate constants of the on-column and the off-column experiments should be done by considering the stationary phase effects that are absent in the off-column experiments.

MODELING THE COMPETITIVE ADSORPTION OF SAMPLE SOLVENT AND SOLUTE IN SUPERCRITICAL FLUID CHROMATOGRAPHY [3]

Competitive adsorption of the solute and solvent was investigated in supercritical fluid chromatography. A series of n-alkylbenzene homologues were chosen as model compounds along with acetonitrile, methanol, and heptane as sample solvents. After a series of preliminary experiments, the phenomenon was success-fully detected with an alkylamide column, at 60◦C temperature,150 bar back pressure and neat carbon dioxide mobile phase. In the case of methanol, the competition was easily identified based on the decreased column efficiency, shifts in retention times and changes in peak widths, since the variation of these properties was highest around the methanol band. Single-component isotherms were determined for methanol and two alkylbenzenes surrounding the solvent band. To account for the adsorption energy distribution of the heterogeneous surface of the stationary phase, the bi-Langmuir isotherm was selected, and the parameters were determined by the inverse method using a numerical method where the differential mass balance equation given by the equilibrium–dispersive model was integrated by the Rouchon algorithm. The results showed a very good agreement between the experimental and calculated band profiles and the behavior of the two different adsorption sites were also explored, all compounds favored site 1 by around two orders of magnitude in terms of the saturation capacity. The competitive bi-Langmuir isotherm was chosen to model the competition. The model employed the determined parameters and a similar numerical approach as before. A series of in silico experiments were performed where all solute concentrations were set in the analytical range and the amount of the solvent was increased step by step to imitate the real injections. Besides the alkylbenzenes, two hypothetical solutes (H1 and H2) were also investigated with varying retentions compared to methanol. Octyl-benzene, decyl benzene and compound H1 were all affected by the displacement effect caused by the strongly adsorbing methanol acting as a displacing agent, resulting in distorted, compressed band profiles and anomalous efficiency. Compound H2 was affected by the tag-along effect caused by the abundance of methanol acting as an inhibitor, resulting in elongated peak shapes and decreased efficiency.

THE USE OF ALTERATION ANALYSIS IN SUPERCRITICAL FLUID CHROMATOGRAPHY TO MONITOR CHANGES IN A SERIES OF CHROMATOGRAMS [4]

Alteration analysis (ALA) is a chemometric method, which provides a unique perspective on an experimental problem. If there is a series of chromatograms and the changes between them are the subjects of interest, our method can provide an easy solution to examine them. ALA can tell where the changes are in the chromatogram, which directions they take and most importantly it can separate different types of changes with synchronous and asynchronous alteration maps. It can differentiate between monotonous and non-monotonous changes and many other among them. ALA's strengths were proven in previous studies through computer generated data. In this study, two practical examples were presented with experimental SFC chromatograms. In the first case, the concentrations of the compounds were perturbed in a similar manner as we discussed in theoretical experiments and the results were also compared against the computer-generated counterpart. Our method delivered the same information in both cases, only the slight retention time shifts made a difference in the experimental series, which means the theoretical properties can be transferred to practice with ease. The second case is about the effect of methanol concentration in the sample solvent. The methanol/acetonitrile ratio was changed step by step from one extreme point to the other and the behavior of the chromato-graphic peaks were monitored and analyzed by ALA. The shift of the location of the methanol peak and its effects on the analyte peaks could be clearly seen on the alteration maps, which provided a unique look on this problem. Correlation maps are also given to compare the novel method to its predecessor and highlight its upgrades.

FLOW-REVERSAL EXPERIMENTS [5,6,7]

Study of the effect of column packing procedure on column end efficiency and on bed heterogeneity

The kinetic performance of a series of 2.1 × 50, 100, and 150 mm columns packed with fully and superficially porous particles were tested and compared. The CORTECS columns provide a slightly greater plate height values at the optimum mobile phase velocity (except of the CORTECS 15 cm column). The flow reversal method was introduced to characterize the sample zone broadening, the intrinsic plate heights, and the differences between the respective column ends. The local plate height values were found smaller with flow reversal than with arrested flow, due to the compensation of the multipath dispersion effect and radial flow profile heterogeneities. We found in every case that efficiency at the respective column ends and the efficiency of the packed bed – i.e. the local plate heights – differ from each other; the columns are heterogeneous, but the difference is negligible, and one can not be sure whether the column inlet or outlet performs better. The local plate height increases with increasing column length. Comparing these local plate heights to the overall column plate heights we can establish the effect of the frits and the column end structure near the frits, which are larger for the CORTECS columns. Furthermore, we can conclude shorter columns can be packed with better bed efficiency. The results show that the column length has an influence on heterogeneity and the shorter the column, the more significant the effect of the frits is. Accordingly, although the packed beds in shorter columns are more homogeneous and the local plate heights are smaller for short than for long columns, the overall efficiency of the short columns is worse than that of the long columns since the relative contribution of the frits is more substantial for short columns.

Measuring the column end efficiency and bed heterogeneity with macromolecules

Peak parking and flow-reversal measurements were extended to macromolecules and carried out with human insulin. We observed that the peaks of insulin are always narrower with reversed flow than without reversing the flow, and the compression effect can be significantly larger than it is for small molecules. The contributions of the column inlet and outlet to the total band variance have been characterized.

The impact of column hardware on efficiency in liquid chromatography

The impact of column hardware on efficiency in LC can be determined via flow-reversal experiments. The dispersion in the frits composes a significant part of the band broadening for modern narrow-bore columns. Therefore, frit technology needs serious improvement, and there are several ways to do that. The frit volume can be reduced by its thickness or the disk-like shape of the frit can be modified from a flat surface to a curved one. As the scanning electron microscopic images demonstrate, the internal macroporous frit structure is heterogenous and disordered. In the near future, 3D-printing technology may offer a solution to this problem.

THE CORRECTNESS OF VAN ’T HOFF PLOTS IN CHIRAL AND ACHIRAL CHROMATOGRAPHY [8]

We have demonstrated that, however, in some cases surprising conclusions can be drawn based on van ’t Hoff plots. The numerical molar thermodynamic values determined from the slope and intercept of the van ’t Hoff plot can be erroneous be- cause of the assumptions made compared to the original van ’t Hoff equation and because of the significant influence of the chromatographic circumstances. Both experimental and theoretical studies show that a more complex thermodynamic study of retention on any type of chromatographic stationary phase is necessary than the one offered by van ’t Hoff plots.

We have created a heterogeneous stationary phase containing two types of adsorption sites by the serial connection of two columns. van ’t Hoff analysis has been used to calculate the thermodynamic parameters for the individual sites and for the heterogeneous surface. It can be concluded that the heterogeneity of the stationary phase made the determination of the accurate ΔH and ΔS values impossible. That observation leads to a serious constraint for van ’t Hoff analysis in chiral chromatography, where stationary phases are intrinsically heterogeneous. Our results also show that pressure drop along the column will strongly influence the calculated enthalpy and entropy values. Therefore, the value of the calculated thermodynamic parameters strongly depends on the length of the column, the particle size, the flow-rate, or the instrument itself.

THE ADSORPTION OF METHANOL ON REVERSED PHASE STATIONARY PHASES IN SUPERCRITICAL FLUID CHROMATOGRAPHY [9]

The results of our study demonstrate that there are very small differences in the surface excess adsorption isotherm for methanol between the two sets of the operational conditions (temperature and back pressure), and carbon dioxide exhibits strong interaction with reversed phase surfaces in both cases. The maximum of the surface excess adsorption for methanol measured with methanol and carbon dioxide mixture as eluent is found at very small set volume fractions 2% and 0.5% (v/v) of the organic modifier in the mobile phase for the ABZ + Plus and Symmetry C 18 columns, respectively. The presence of polar groups such as amide in the structure of bonded ligands increases the hydrophilic properties of the surface which causes the increase of methanol sorption by hydrogen bonding. The surface heterogeneity may have an important influence on solvent adsorption, consequently on the separation selectivity. Besides the adsorption study of methanol as a modifier, the effect of methanol and heptane as sample solvents for the alkylbenzenes sample have been investigated. In case of methanol, the peak efficiency of solutes is increased because of the displacement effect of methanol, then the efficiency is decreased suddenly (around the methanol peak) because of the tag-along effect caused by the abundance of methanol which hinders the solutes to reach the adsorption sites.

THE IMPACT OF PLACEMENT, EXPERIMENTAL CONDITIONS, AND INJECTIONS ON MASS FLOW MEASUREMENTS IN SUPERCRITICAL FLUID CHROMATOGRAPHY [10]

Our work demonstrates that even though mass flow rate is the only flow parameter considered constant in SFC, some variation can be still expected when taken at different parts of the chromatographic instrument, since the CFM alters the system configuration. Comparing mass flow rates between the inlet and outlet of the columns showed diverse tendencies in differences ranging from 0.6% to 4.2%. Considering that only neat CO_2 was used as mobile phase in the study, deviations were not too severe. In the case of mobile phases containing organic modifier and additives as well, even lower differences should be expected. Pressure measurements complementing the work showed varied pressure drops on the columns depending on their length and particle size. Interestingly, significant pressure drops were found on the mass flow meter, more pronounced at the inlet side (1.5–2 bar), that suggest a slight effect on mobile phase flow. Studying the effect of pressure and temperature on mass flow- rate showed that the former had a larger influence while changing temperature only had minimal effects. Accounting for injections showed that although the initial drop in mass flow is severe compared to the equilibrium, taking the average from the injection time until the hold-up time reduced this effect significantly. The use of well-retained compounds should further minimize the adverse effect of injections. Precision studies revealed that measuring accurate, reproducible mass flow-rates in a low-flow, low-viscosity environment is problematic in a standard laboratory setup even if the built-in self- diagnostics of the SFC system show no leaks, the CFM calibration is correct and all instructions are strictly followed.

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