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Development of separation systems for multi-dimensional liquid chromatography

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Liquid chromatography (LC) is an important technique for separating (complex) mixtures of non-volatile analytes. By combining two LC phase systems with independent ("orthogonal") retention mechanisms gains in separation power and analysis time can be achieved. By adding a third orthogonal separation mechanism the gain in separation power can be enormous. In this thesis progress is described in creating stationary phases suitable for implementation in a three-dimensional spatial LC (3D-LC or LC×LC×LC) device. The investigated retention mechanisms that may potentially be combined include iso-electric focussing, ion-exchange LC and reversed phase LC (RP-LC).

In *Chapter 2* a procedure is described to create a strong-cation-exchange (SCX) column via a photo-chemically-induced post-polymerization reaction on an RP monolithic stationary phase. Photografted SCX columns were implemented in a two-dimensional system (LC×LC) in combination with a non-grafted RP-LC stationary phase. This LC×LC system was hyphenated with a high-resolution Fourier-transform ion-cyclotron-resonance mass spectrometer (FTICR-MS). With the resulting LC×LC-FTICR-MS system separation and identification of tryptic-digested proteins were achieved.

In proteomics, samples covering a large range of analyte concentrations are encountered. Such analysis demands a high loadability and, thus, a relatively large internal-diameter (^1d_c) of the first-dimension (^{1}D) column. MS sensitivity, in contrast, typically increases when miniaturizing the ionization source, *i.e.* when decreasing the second-dimension (^{2}D) column diameter (^2d_c). In LC×LC the fraction transferred from the ^{1}D to the ^{2}D

column needs to be as small as possible, so as to minimize injection band-broadening effects in the ^{2}D and maintain a good performance. Hence, there is a conflict between the requirements of efficient LC×LC separations ($^{2}d_c$ >> $^{1}d_c$) and those of sensitive LC×LC-MS analyses (small $^{2}d_c$ and $^{1}d_c$ >> $^{2}d_c$).

In *Chapter 3* this LC×LC paradigm is addressed by implementing active modulation. A retentive material was used to trap the analytes during collection of the ^{1}D effluent, which meant that the injection band broadening on the ^{2}D column could be reduced. This modulation also allowed us to desalt the ^{1}D effluent on-line, prior to injection on the ^{2}D column. Active modulation makes it possible to combine virtually any pair of columns. The applicability of this approach was demonstrated by combining a ^{1}D SCX column with an 0.3-mm internal-diameter (i.d.) with an a 0.075-mm i.d. ^{2}D RP-LC column in an LC×LC-FTICR-MS system for the proteome-wide analyses of a *Saccharomyces cerevisiae* digest.

In the second part of this thesis the experiments are described that involved titanium-scaffolded monolithic columns. The idea behind this scaffolding was to reduce the overall shrinkage of an organic-monolithic stationary phase in a large conduit upon polymerization and upon changing the mobile-phase composition during (*e.g.* during gradient runs).

In *Chapter 4* "narrow-bore" scaffolded square conduits (1.3×1.3 mm) are described. Monolithic stationary phases were created inside these scaffolded columns and they were subsequently used for the separation of intact proteins. The chromatographic performance was stable, under conventional as well as under ultra-high-pressure (UHPLC) conditions with pressure drops exceeding 40 MPa.

In *Chapter 5* a large-internal-diameter titanium-scaffold (4.0×4.0 mm square conduit) is described, which was designed to demonstrate the possibility to adequately control the temperature under UHPLC conditions.

In the *Chapter 6* various experiments are described that were performed to study separation mechanisms that may be suitable for incorporation in a spatial chip device. These include electrophoresis, ion-exchange chromatography and reversed-phase LC. Finally, suggestions are made to progress towards the development of a spatial 3D-LC device.