Optimization of Designs for Spatial Multi-dimensional Separations

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Summary

Scientists are creating many needs to study an increasing number of samples of increasing complexity. This results in mounting demands for analytical separation techniques, of which liquid chromatography (LC) is one of the major exponents. The efficiency of chromatographic systems has become an essential factor for the advancement of scientific research. Comprehensive two-dimensional column liquid chromatography (^{t}LC×^{t}LC) constitutes a step forward. In comparison with conventional one-dimensional LC the separation power (in terms of the peak-production rate, *i.e.* the peak capacity per unit time) can be increased by one to two orders of magnitude for complex separations. A different approach for two-dimensional liquid chromatography is based on separation in space (^{x}LC×^{x}LC). In time-based separations analyte are separated based on their time of elution from a chromatographic system. In space-based separations they are separated based on their position in the separation medium. If we extend spatial separations to three dimensions (^{x}LC×^{x}LC×^{t}LC) the separation power can be increased quite dramatically. The aim of this thesis is to investigate the potential of different modes of multi-dimensional LC systems and to formulate guidelines for the optimal design of spatial separation devices. *Chapter 1* provides an introduction to multi-dimensional liquid chromatography.

In *Chapter 2* a Pareto-optimization approach is applied to a number of realistic combinations of space- and time-based separations for three-dimensional systems. The efficiency of these systems is evaluated in terms of achieving the maximum peak capacity in the minimum analysis time. The eye-catching result of these calculations is that a peak capacity of several hundreds of thousands of peaks may be achievable within a few hours using an ^{x}LC×^{x}LC×^{t}LC system under moderate conditions (5 MPa). In an overnight run (16 hours) a peak capacity of one million appears to be within reach.

In *Chapter 3* microfluidic chips are considered that feature one first-dimension (^{1}D) separation channel, 16 second-dimension (^{2}D) channels, and 256 or 1024 third-dimension (^{3}D) channels with specified lengths. The maximum achievable performance is calculated, again using a Pareto-optimization approach. In such a system the performance of the "ideal" device of *Chapter 2* is not approached. However, the performance of column-based ^{t}LC×^{t}LC systems for the separation of proteins is expected to be considerably exceeded. A monolithic stationary phase has been synthesized *in-situ* in a microfluidic chip.

In *Chapter 4* design aspects of two main types of flow distributors – radially interconnected (RI) and bifurcating (BF) – are discussed based on computational fluid dynamics (CFD). Guidelines are formulated for designing such flow distributors and the effects of imperfections or blockages are studied.

In *Chapter 5* the theoretical approach described in *Chapter 4* is validated in practice, using chips fabricated by micromilling. CFD simulations of the distribution of analytes in two-dimensional flow systems are presented. Flow confinement within the ^{1}D channel by applying flow resistance to the segments near the outlets of the flow distributor is demonstrated by CFD simulations. The presence of constrictions for the same purpose is also evaluated by CFD and the results of these simulations were confirmed experimentally.

Chapter 6 contains some reflections on the development of microfluidic devices for multi-dimensional separations. Possibilities of various designs and of implementing different chromatographic supports are discussed.