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Single-electrolyte isotachophoresis : on-chip analyte focusing and separation

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Conclusions and Perspectives

Analysis of a wide range of biomolecules such as metabolites and peptides especially in small sample volumes is still a major challenge in analytical chemistry in general, and areas such as metabolomics especially. This thesis has presented novel concepts and methods for single-electrolyte isotachopheresis (ITP) using electric field gradient focusing (EFGF) in general and concentration polarization-based devices in particular. These concepts and methods can have a major impact on novel analytical workflows for the analysis of charged biomolecules.

In chapter 2, ITP and EFGF principles and methods have been reviewed. It has been argued that ITP and EFGF are identical types of methods because they share the isotachopheretic principle: all focused ions are moving with the same velocity. It is discussed that isotachopheretic phenomena are indeed ubiquitous in the wide range of EFGF methods that have been previously published. It is therefore proposed that the strengths of ITP and EFGF can therefore be combined to offer new opportunities in the sample preparation of small samples, and for the bioanalysis of complex samples in general.

Future challenges for ITP/EFGF methods include improvement of reproducibility and robustness, extensive characterizations, interfacing with appropriate detection technologies, such as mass spectrometry or detectors with a minimal size, downscaling and integration on microfluidic chip

platforms. This will result in ITP/EFGEF-based biochemical assays for diagnostics.

In chapter 3, depletion zone isotachopheresis (dzITP) in an H-shaped micro/nanofluidic glass devices has been described. By focusing analytes at the border of a nanochannel-induced depletion zone, isotachopheresis could be performed using a single electrolyte only. Three-point voltage actuation controlled the extent of focusing and the positioning of isotachopheretic analyte zones, a feature which may be useful for repeated scanning along a point sensor. As a single-electrolyte method, dzITP is an important simplification over conventional ITP. This could become particularly beneficial in designing simple to operate point-of-care devices.

In chapter 4, it has been shown that tunable ionic mobility filtering can be added to the functionality of dzITP. Through voltage- and/or current actuation, the release of compounds along the depletion zone has been controlled, the depletion zone being comparable to a valve that can be opened or closed at will. Both pulsed and continuous operation modes have been demonstrated. Using simultaneous dzITP filtering and focusing, a low-concentration compound was specifically enriched, while a second, high abundant compound was continuously released. Moreover, specific high-mobility compounds from diluted raw urine sample were trapped in isotachopheretic zones while low-mobility compounds were released. Fluorescein was used as a marker both for ionic mobility cut-off and for indirect detection. These experiments show the potential of dzITP for the

selective enrichment of low-abundant analytes in the presence of high concentrations of matrix compounds.

In chapter 5, a PDMS concentration polarization device was developed using an elastomeric microvalve to create a tunable reversible nanospace in a microchannel. Measurements of valve resistance indicated nanospace heights between ~7 and ~60 nm that could be obtained as dependent on valve pressure. By voltage and valve pressure regulation, a wide range of concentration polarization regimes was achieved. A more than 1000-fold pre-concentrated compound was released by opening the valve. Though not demonstrated in this thesis, ITP separations might evolve when multiple compounds are focused at the border of a valve-induced depletion zone. In this way, PDMS microvalve devices might be used as single-channel dzITP devices.

From the insights and results reported in this thesis, it is clear that single-electrolyte isotachopheresis has enormous versatility. Efficient pre-concentration, separation, filtering, compound selection, zone positioning, and indirect detection are all possible in a single method. It is expected that several operations may be added to this palette, including derivatization reactions, buffer replacement and desalting. These operations will not only be available for dzITP in both glass- and PDMS-based devices, but also to many of the other members of the large EFGF family.

The methods presented in this thesis are all chip-based. A key advantage is that in principle only very small sample volumes are needed. For discrete

dzITP injections as described in Chapter 2 and 3, injection volumes were 0.3 nL. Another important aspect is the potential for massive parallelization. It is expected to be possible to integrate hundreds of dzITP channels on a single chip. The very small footprint of the device is advantageous in this regard. For example, the device shown in Chapter 2 and 3 requires an area of 0.1 x 2.0 mm only (connections to fluidic reservoirs excluded). For discrete injections, a focused zone in dzITP may have a volume of less than 10 pL. We also envision on-chip integration of dzITP modules with other modules, for example reagent mixing or detection modules.

Future research should focus on dzITP assay development and on combining dzITP with electrospray ionization-mass spectrometry (ESI-MS). ESI-MS is a powerful tool for sensitive detection which provides rich information about chemical composition. Combined with ESI-MS and possibly with the great separation power of capillary zone electrophoresis, dzITP will greatly extend the coverage of comprehensive analyses of complex biological samples.