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Capillary electrophoresis-mass spectrometry based metabolomics approaches for volume-restricted applications

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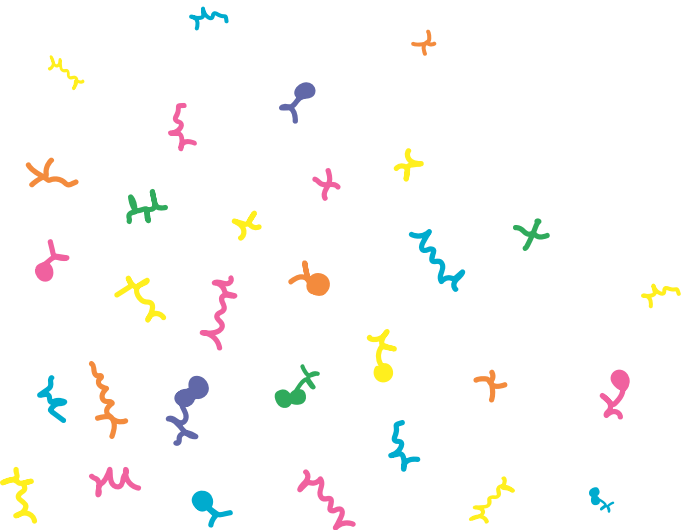

Chapter 7

Concluding remarks and perspectives

The ultimate aim of metabolomics research is to obtain an answer to a well-defined biomedical or clinical question. At present, the analytical techniques commonly employed for metabolomics studies fall short for addressing volume-restricted biological questions. Therefore, in this thesis, the key aim was the development of reliable and sensitive microscale analytical methods and workflows in order to enable the study of questions intrinsically dealing with low sample amounts. To realize this ambition, capillary electrophoresis-mass spectrometry (CE-MS) was selected as analytical technique given its potential to separate a wide range of (polar) ionogenic compounds in tiny sample amounts with a high separation efficiency and selectivity, in order to show that CE can be made as suitable as LC-MS for metabolomics. At the start of this thesis, various CE-MS methods had been developed for profiling (endogenous) metabolites in small-volume biological samples in the years prior to the start of the thesis. However, the studies reported mainly focused on showing the proof-of-principle of CE-MS for this purpose. The work described in this thesis can be considered a seamless follow-up of the prior art with a special emphasis on showing the actual applicability of CE-MS-based workflows for volume-restricted metabolomics studies. More specifically, the utility of new or refined CE-MS workflows is shown for both targeted, quantitative analysis and for non-targeted profiling of endogenous metabolites in low amounts of biological sample in order to answer relevant biological questions. With the established methods, we focused on the profiling of (highly) polar ionogenic metabolites in different material-limited matrices that are of interest for neuroscience research, such as brain fluids, low amounts of HepG2 cells and zebrafish larvae models. A major goal was to demonstrate that the technical reproducibility, detection sensitivity and metabolic coverage of CE-MS are sufficient to allow use in biomedical applications, and current misconceptions about these in CE-MS are not valid anymore [38]. All of these aspects will be discussed in the following sections, including some remaining challenges and future perspectives.

Conclusions

Reproducibility of CE-MS for metabolomics

One of the most important aspects in metabolomics studies is the reproducibility of an analytical method, which remains a critical factor in cross-laboratory comparative studies using heterogeneous instruments. In this regard, the migration time (or retention time for chromatographic techniques) is an important parameter as it ensures a reliable comparison of metabolic profiles, including examining samples for subtle changes in patterns. Even though a well-known drawback of CE-MS is the variability in migration time between runs, the conversion of migration times to effective electrophoretic mobilities has already shown to be highly beneficial towards reliable metabolite identification in a repeatable manner [137]. At

the beginning of this thesis, the actual reproducibility of CE-MS using effective electrophoretic mobility as the key parameter for compound identification was further evaluated in an interlaboratory study. As demonstrated in **chapter 3**, the Metabo-ring study included 16 CE-MS systems, divided over 13 independent laboratories from 11 countries, with high instrumental and methodological heterogeneity between the participating labs. The only prerequisite for the trial study was the preparation of the acidic BGE according to a protocol.

In **chapter 3**, we show that the conversion of migration times into effective electrophoretic mobilities resulted in a variability of 3.1% compared to a variability of 10.9% when using the relative migration time scale, while employing the same BGE composition. The preparation of the BGE was found to be crucial in order to acquire reproducible results for the effective mobility, as especially the electrophoretic mobilities of slow-migrating compounds are heavily influenced by slight changes in the acidity of the BGE. This stresses the importance of standardized protocols for BGE preparation when performing (comparative) metabolomics studies, especially for less experienced users of CE-MS.

The Metabo-ring study addresses the main weakness of the commonly used relative migration time (RMT) approach, as the RMT is still influenced by fluctuations of the electroosmotic flow (EOF). We found that the RMT approach can only be used in a reliable fashion for compounds with a similar migration velocity as the internal standard, but increases in bias for compounds that migrate significantly faster or slower (up to 40% bias). This indicates that in order to reliably compare interlaboratory RMT values, multiple internal standards with different migration velocities need to be included in the analysis. In contrast, migration time correction using electrophoretic mobility showed similar bias (<5%) over a broad migration time range using merely two internal standards, due to a neutral marker that corrects for the EOF mobility. This shows the strength of using electrophoretic mobility as a parameter for direct interlaboratory data comparison, but also for metabolite identification using effective mobility libraries. Moreover, the ability to directly compare effective mobilities between different labs regardless of instrumental and methodological heterogeneity shows the advantage of CE-MS for global metabolomics compared to another commonly used analytical technique for the analysis of polar metabolites i.e. hydrophilic interaction liquid chromatography (HILIC)-MS. When using HILIC-MS, there is often a lack of standardization in operating parameters such as the column material, the injection solvent and the (preparation of) mobile phase that causes variable separation conditions, which makes interlaboratory comparison of retention times tedious.

Overall, the CE-MS Metabo-ring study revealed the potential of using the effective electrophoretic mobility as a universal and reliable criterion of peak identification in metabolomics. Even though this study predominantly focused on the profiling of cationic metabolites, it is anticipated that the same strategy could also be applied to anionic metabolic profiling. However, the robust and sensitive analysis of anionic metabolites has proven to be quite challenging, as is discussed in **chapter 5**.

Sensitivity enhancement

The prerequisite of low sample volumes for analysis makes CE-MS an attractive tool for the analysis of volume-limited samples. Nevertheless, due to the limited loading capacity of CE and concentration-sensitive detection of ESI-MS, CE-MS is often perceived as non-suitable for trace metabolomics [38]. However, the loading capacity of CE has been addressed effectively by the use of in-capillary sample preconcentration techniques. In **chapter 4**, we present an in-capillary preconcentration procedure with sample stacking based on dynamic pH junction to profile endogenous metabolites in rat brain microdialysis samples. The preconcentration was optimized systematically by response surface design (RSM), and allowed for a sample injection corresponding of about 291 nanoliter (compared to the typical 18 nanoliter).

The method was used to directly analyze microdialysates in a highly repeatable way employing minimum sample pretreatment (1:1 dilution in BGE). This limited sample preparation is especially beneficial when dealing with very low sample volumes, as an increased number of sample preparation steps may increase the bias due to handling errors and metabolite loss. Detection limits were in the low nanomolar range for amino acid neurotransmitters, already showing the methods potential for trace-sensitive brain metabolomics studies. Moreover, CE-MS could definitely yield comparable detection limits as compared to conventional LC-MS methods, with the main difference being that for CE only a volume of about 30-300 nL is injected, while 5-10 µL in the sample vial is sufficient for injection, whereas typically 300-10000 nL is injected in a conventional LC-MS method. This makes using CE-MS beneficial when the sample amount is very limited.

In this thesis, we employed the CE-MS method to analyze 48 endogenous compounds in rat brain microdialysis samples, of which 25 metabolites were (provisionally) identified and quantified. These metabolites include amino acids and related compounds, which are associated with glutamatergic, GABAergic and glycinergic systems, which play a key role in the pathophysiology of many neurological and neuropsychiatric diseases, such as epilepsy [338] and major depressive disorder [339]. The ability of our method to provide absolute quantification of these metabolites from different time points of *in-vivo* brain extracellular fluid samples

demonstrates the potential of CE-MS to gain better understanding of the underlying neurochemistry of neurological diseases, including how treatments might regulate these causes. Additionally, besides rat brain microdialysis samples, this method could also easily be implemented to directly profile a range of other low-volume high-conductivity sample matrices including tears, sweat or brain microdialysates from smaller animal models such as mice.

In this study, a volume of 10 µL was present in the sample vial to ensure reliable sample injection. However, when only nanoliters are injected into the CE system from the sample vial, the volume mismatch may limit the analytical performance of CE-MS for material-limited metabolomics studies. The development novel interfacing designs, the modification of sample vials [340, 341] or the use of mineral oils [342], allow for even lower sample volumes in the sample vial (1-2 µL). To apply direct sample injection with sample stacking on such an adapted system would be highly beneficial for brain microdialysis studies, where an improved temporal resolution is an important factor in order to profile neurotransmitters and neuromodulators over time.

Improved metabolic coverage

For cationic metabolic profiling, well-established CE-MS protocols have been developed and employed to analyze large sample cohorts. On the other hand, the development of a robust CE-MS approach for anionic metabolic profiling is still an ongoing development. Over the last decade, efforts have been made to develop analytical techniques for the selective and sensitive analysis of acidic ionogenic metabolites. In **chapter 5A**, an overview is provided of the recent methodological developments in this specific research area. Based on the examination of reported literature, we concluded that there is still room for the development of a highly sensitive and reliable CE-MS method for anionic metabolic profiling. Therefore we investigated the use of chemical derivatization to order to profile anionic metabolites in low amounts of HepG2 cells. In **chapter 5B**, we introduced a pre-column derivatization CE-MS method for the analysis of anionic metabolites using exactly the same conditions as employed for the analysis of basic metabolites. In this method, we use a novel chemical derivatization reagent; trimethylmethaneaminophenacetyl bromide (TmAmPBr), which is an adaptation of the commercially available dimethylaminophenacyl bromide (DmPABr) reagent that has already been successfully employed to label primary amines, secondary amines, thiols and carboxyls in (RP)LC-MS workflows [95, 290]. The tertiary amine group of DmPABr is replaced by a quaternary amine group in TmAmPBr to ensure a permanent positive charge on the derivatives, which is beneficial in terms of ionization efficiency and minimizes ion suppression in the ESI source. The original

reaction conditions from Willacey *et al.* [95] were adapted and altered to ensure reliable derivatization in presence of the quaternary amine, and to improve detection sensitivity in CE-MS. The derivatization strategy was further optimized using RSM.

The optimized method provided detection limits in the mid- to low nM range for a range of carboxylic acid containing metabolites, showing a 10-100 times sensitivity improvement compared to the state-of-the-art CE-MS methods that use negative ionization mode. Therefore, this work demonstrates that CE-MS with the use of pre-capillary TmMPABr derivatization of small organic acids can compete with the latest GC-MS and RPLC-MS techniques when it comes to sensitivity. To showcase its applicability for low-volume biological samples, the method was employed to profile organic acids in low amounts of HepG2 cells (ranging from 5000 to 2.5 × 10^5 cells). This shows the suitability of the method to study a range of diseases associated to abnormalities in tricarboxylic acid cycle (TCA) metabolites [343].

Based on the similar reaction properties compared to the DmPABr reagent, it is anticipated that the novel derivatization procedure can be further expanded to a wider selection of metabolites, potentially even allowing the analysis of both acidic and basic metabolites in a single CE-MS run using the same separation conditions. However, this requires further evaluation of labelling patterns and potential competition between functional groups.

Overall, this proof-of-principle study shows the potential of CE-MS with pre-capillary derivatization to study TCA cycle and energy metabolism in material limited samples. However, before the method can be used for actual metabolomics research, the aspects discussed above require some further attention.

The applicability of CE-MS biomarker discovery

The utility of the CE-MS workflows developed in **chapters 4 and 5B** is shown for targeted metabolomics, thereby providing absolute quantification of a chosen subset of metabolites related to neurological and neurodegenerative diseases, i.e. amino acids in **chapter 4**, and organic acids in **chapter 5B**. This approach takes advantage of the comprehensive understanding of a vast subset of metabolites and the known biochemical pathways to which they contribute. On the other hand, non-targeted metabolomics involves global profiling of the metabolome, and allows us to compare metabolic profiles in diseased groups and healthy control groups in order to gain a better understanding of the mechanism of disease onset and progression. Therefore, in this thesis, we also investigated the applicability of CE-MS to perform non-targeted metabolomics.

In **chapter 6** we demonstrate a non-targeted metabolomics workflow for the profiling of endogenous metabolites in extracts from individual zebrafish larvae and pools of small numbers of larvae. We utilized the CE-MS conditions first presented in **chapter 3** (10% acetic acid as BGE), and devoted special attention to an efficient and effective sample homogenization and extraction strategy. Zebrafish larvae pools with lowering numbers of larvae per pool (40, 20, 16, 12, 8, 4, 2 and 1 larva) were analyzed, yielding 84 metabolites detected for 40 zebrafish, and still 29 detected metabolites in a single zebrafish larvae. This already shows the potential of CE-MS to perform metabolomics studies in one single zebrafish larva. Additionally, when a stacking procedure as presented in **chapter 4** is used, even more metabolites can be detected in a single larva. For metabolite identification purposes, we used electrophoretic mobilities and compared these to a library containing over 600 metabolite entries [137]. As an extra layer of annotation confidence, two different fragmentor voltages (100V and 200V) were applied to induce in-source fragmentation using a TOF-MS instrument [311].

The applicability of the method to differentiate metabolites between groups was demonstrated by using a zebrafish larvae stress model. For this purpose, wildtype and ubiquitous mineralocorticoid (MR)-knockout zebrafish larvae were used, which were exposed to an exogenous cortisol treatment. Up to now, it has been barely investigated what the specific role is of MR during stress on metabolite levels, and which mechanisms might be important in this association. Using multivariate data analysis, different metabolic profiles could be obtained for all four groups, clearly indicating that MR has a role in stress response. This preliminary work shows the potential of our CE-MS workflow to be used in neuroendocrinology studies, which encompasses the study of the interactions between hormones and the brain. An interesting next step for this research would be to include zebrafish larvae behavior, and to investigate how hormones such as cortisol affect the metabolome and related behavioral changes.

It should be noted that due to its great diversity and complexity, it is impossible to analyze the complete metabolome within a biological system using a single analytical technique, and therefore often multiple analytical techniques are employed to acquire maximal biochemical information. In that regard, it would be interesting to perform multi-platform metabolomics on the zebrafish stress model, thereby including both polar and non-polar metabolites. This would be possible when using for example a two-phase extraction strategy (i.e. Bligh and Dyer extraction), and analyze the aqueous layer using CE-MS, and the organic layer using RPLC-MS or NACE-MS. This will provide a more comprehensive view on the metabolome of zebrafish larvae when exposed to stress.

Future perspectives

Further improving identification capability

As for now, electrophoretic mobility is still rarely used as a direct parameter of metabolite identification. However, a recently developed software tool that converts the migration-time scale into an effective electrophoretic mobility scale, designated as ROMANCE [152], might further encourage research groups to start using effective mobilities as additional parameter for metabolite identification in their analyses, either via targeted or non-targeted metabolomics. The development of such free, easy to use software has already led to the construction of compound libraries that include electrophoretic mobility values under specific BGE compositions [137]. When electrophoretic mobilities are added into universal databases such as the Human Metabolome Database (HMDB) [55] or METLIN [56], another compound-dependent and reliable value will be provided, thus complementing accurate mass and fragmentation patterns. Additionally, electrophoretic mobilities are easier to use in order to predict new molecules than retention time in the case of HILIC-MS.

Another interesting property to improve metabolite identification in CE-MS is collision cross-section (CCS), which was introduced with the development of ion mobility (IM-)MS. IM-MS adds an extra dimension of separation based on conformational differences, making it particularly interesting for the analysis of compounds that are typically not (well) separated by CE [286, 344]. Even though this is not widely used for metabolomics studies yet, we anticipate that IM-MS will be more extensively applied for metabolomics in the near future.

The importance of standardized procedures

The lack of standard operating procedures and data workflows that are fit for purpose may have hindered the widespread use of CE-MS in metabolomics. Relevant technological and practical aspects for the metabolomics workflows presented in **chapters 4 and 5B** are highlighted in detailed protocol papers [345] and [346]. Besides written protocol papers, an essential recent development the availability of peer-reviewed video articles [162, 347-349]. The availability of standard operating procedures ensuring accurate and reproducible results would be highly favorable in order to encourage researchers to actively consider CE-MS for metabolomics studies.

Furthermore, the recent CE-MS Metabo-ring trial showcased in **chapter 3** clearly revealed that this methodology can be used in a relatively simple way, even by groups that do not have (any) experience with metabolomics research.

Multivariate optimization for analytical workflows

As mentioned earlier, the workflows in **chapters 4 and 5B** were optimized in a systematic way by employing RSM. RSM is a Design of Experiments (DoE) that entails a multivariate optimization tool that encompasses mathematical and statistical techniques in order to study the relationship between multiple input variables (factors) and key output variables (responses). In comparison to the classical one-variable-at-a-time (OVAT) approach, RSM allows for the simultaneous evaluation of multiple factors during method optimization. A major advantage of RSM over OVAT is the reduced number of experiments, saving both time and experimental resources. Additionally, as is showcased in this thesis, RSM allows to investigate the interaction between factors to get a deeper understanding of a process and identify which factors have the largest influence on the response. In contrast, the interaction effects would most likely been overlooked when using the traditional OVAT approach, as it is highly influenced by the order in which the factors are optimized. The two presented studies in this thesis show that RSM is a powerful and efficient tool for method optimization in metabolomics.

However, RSM is not yet widely used in research labs, mainly because it is perceived as a complex methodology that requires deep understanding of statistics and mathematics. Furthermore, elements such as selecting a proper design are critical in obtaining the optimal characterization of a process. Therefore, it is important that general guidelines are provided on how to design DoE and how to interpret the acquired data. A recent study tested over multiple DoE strategies incorporating over 500.000 experimental simulations [350], reported their findings and provided general recommendations. The development of these guidelines, together with easy-to-use statistical software packages and tutorials may encourage the widespread use of RSM in the near future.

Quantitative performance of CE-MS

Besides its qualitative performance, the ability of an analytical technique to quantify metabolite concentrations in a reliable manner is of upmost importance for metabolomic studies. For non-targeted metabolomics studies, typically a relative quantification strategy is sufficient to capture changes in the metabolome after perturbations to the biological system in question. On the other hand, for targeted metabolomics studies, absolute quantification of metabolite concentrations is often preferred. In MS-based targeted metabolomics workflows, attention should be paid towards (standardized) sample preparation strategies to minimize ion suppression and improve the quantitative accuracy. Furthermore, the addition of (isotope labelled) internal standards for peak area correction is essential in targeted quantitative metabolomics. However, especially isotope labelled standards can be

very costly, and in practice often only a few internal standards are used to correct for a range of metabolites, as is for example demonstrated in **chapter 4,** where 5 internal standards were used to quantify 25 metabolites. A great potential of using a derivatization approach as described in **chapter 5**B is the use of isotope-coded-derivatization. This relatively easy and cost-efficient strategy allows the addition of a stable-isotope-labelled internal standard for every metabolite, as for example shown by Willacey *et al*. [95].

Recently, Harada et al. demonstrated the quantitative performance of CE-MS through the analysis of over 8000 human plasma samples [270]. However, before CE-MS could be adapted for reliable absolute quantification in a large-scale (routine) clinical setting, we believe it is necessary to set up an additional interlaboratory study to assess the quantitative performance of CE-MS.

Electrokinetic-based preconcentration

As has been demonstrated in **chapter 4**, significant sensitivity enhancement can be achieved relatively simple and fast by (temporarily) manipulating the electrophoretic mobility of metabolites. Besides dynamic pH junction, another promising in-capillary preconcentration strategy for the analysis of low concentrations of metabolites in low-volume samples is electrokinetic supercharging (EKS). EKS is based on electrokinetic injection and combines the preconcentration techniques of field-amplified sample injection and transient isotachophoresis. Wells *et al*. applied EKS for neurotransmitter analysis in volume-limited tissue samples from rat brain tissue and whole Drosophila [112], thereby reaching detection limits as low as 10 picomolar, showing a 1000 times improvement compared to pH junction. These detection limits show the potential of this EKS for profiling of low-abundant catecholamine neurotransmitters such as dopamine and serotonin, which are already important targets for therapeutic drugs. However, even though EKS could yield very low detection limits, its application in actual biological samples is still limited. This is probably because the relatively low repeatability and low recovery of EKS due to the injection bias of electrokinetic injection as a result of varying salt concentrations. Therefore, EKS is not directly applicable to high conductive sample matrices. Moreover, the effectiveness of EKS is significantly higher for metabolites with a high electrophoretic mobility such as lysine or GABA, and shows hardly any sensitivity improvement for low-mobility metabolites such as aspartic acid or inosine. Nevertheless, it is still an interesting procedure to further study and optimize, by for instance adding a desalting step as recently reported by Liao *et al*. [351] prior to electrokinetic sample injection to improve injection repeatability.

Another interesting electromigration based preconcentration strategy is electroextraction (EE). Instead of an in-capillary preconcentration strategy, EE is performed prior to sample injection and takes place in an immiscible liquid-liquid system consisting of an organic phase and an aqueous phase. Extraction takes place from the donor phase to the acceptor phase through the application of an electric field, either with (electromembrane extraction, EME) or without a filter phase (EE). Over the last years, EE and EME have been coupled on-line to various analytical techniques, showing its potential for automated high-throughput analysis of low-volume biological samples [352]. Recently, Oedit *et al*. developed a fully automated two-phase electroextraction system coupled on-line to CE-MS for the analysis of urine samples [353], which resulted in low nanomolar detection limits. The on-line coupling of EE to CE-MS allows to utilize a sample volume of merely 300 nanoliter, greatly lowering the amount of sample needed for analysis, thereby showing its potential for implementation in chip-based systems. It should be noted, that as with the electrokinetic procedure described above, also for EE the salt concentration in the sample plays an important role in its efficiency.

To further improve the sensitivity of the method presented in **chapter 5**B and reach detection limits that allow single cell analysis, it would be interesting to explore electrophoretic-based preconcentration strategies as discussed above for this purpose. The high selectivity of electrokinetic injection would be highly beneficial in combination with the permanent positive charge on the derivatives, even further lowering ionization suppression caused by (uncharged) matrix.

Alternative interfacing designs

In this thesis, a conventional, commercially available co-axial sheath-liquid interface was used for coupling CE to MS. The sheath-liquid interface has proven itself as a robust system for metabolomics, and has shown its applicability the last few years for large-scale metabolomics studies in urine- [354] and plasma samples [270, 355, 356]. However, employing a sheath-liquid interface leads to sample dilution at the ESI sprayer tip, causing a reduction in sensitivity. As a result, over the past few years some significant developments have been made in CE-MS interfacing techniques [108, 262].

An important event was the introduction of the sheathless porous tips based interface by Moini in 2007 [252], which has already proven itself the last few years for metabolomics analysis of material-limited samples [340, 357]. As an example, Zhang *et al*. [273] developed a sheathless CE-MS method for the profiling of nucleotides in an extract of only 500 HepG2 cells, corresponding to less than a single cell injected onto the capillary. Nevertheless, the porous tip has not yet been adopted as a routine CE-MS method, which is mainly due to the

complexity of system handling and the lack of capillary-to-capillary repeatability. Another interfacing trend is lowering the flow of the shealth-liquid interface. The use of an alternative CE-MS interfacing design was recently shown for single cell analysis. Lombard-Banek *et al*. reported a CE-MS method that allowed *in-vivo* single-cell proteomics and metabolomics in the same single cell in chordate embryos using X. *laevis* [269]. With a custom-build CE-μESI platform, quantitative proteo-metabolomic differences were observed between cells at the cleavage stage. This shows the potential of alternative CE-MS interfacing designs for single cell analysis, thereby including the ability to study cell heterogeneity in future metabolomics studies. However, special attention needs to be paid towards the efficient sampling and sample preparation of these minute sample amounts, as this is the most challenging and demanding process in single cell metabolomics [358].

Translation to the clinic

For clinical metabolomics, the high-throughput analysis of dozens, hundreds or even thousands of biological samples is important. In particular, there is a requirement for fast and robust metabolomics workflows for material-limited samples. An important development in CE-MS analysis strategies is the multi-segment injection (MSI) approach [227], presented in 2013 by the research group of Britz-Mckibbin. MSI allows for serial injections of multiple samples within a single capillary, thereby greatly improving the sample throughput. Furthermore, when including a quality control (QC) sample in the same run, stringent quality control and batch correction can be performed. Recently, MSI-CE-MS potential for large-scale metabolomics was shown in a study including over a thousand serum samples [308], where metabolic fingerprints in serum samples from pregnant woman all over Canada. The samples were analysed for 7 months using standardized methodology and data treatment, and the results showed acceptable intermediate precision for a range of metabolites. Overall, this study demonstrated the value of MSI-CE-MS for performing large-scale high throughput metabolomics studies in a repeatable manner, thereby including successful correction for long-term signal drift and inter-batch variations.

A potential field where CE-MS could be especially useful is neonatal healthcare. Early and definitive diagnosis is critical for the clinical management of newborns and infants with inborn errors of metabolism or infections. Current newborn screening programs still face some challenges, such as high false positives and delayed diagnosis and an inability to screen for certain metabolic disorders. Another aspect requiring further research is using the volume-restricted material from newborns more effectively for screening purposes. During the end-phase of this thesis, we performed a pilot study where we demonstrated the utility of

sheathless CE-MS employing MSI for profiling of endogenous metabolites in volume-restricted serum samples from neonates. The study aims to assess the utility of CE-MS by determining creatinine levels in plasma collected from neonates at a clinical lab and comparing these findings with the assay used routinely in the clinical chemistry lab for this purpose.

In summary, in this thesis, with our developed methods we have shown the versatility of CE-MS and its applicability for a number of different sample matrices i.e. brain fluid, cells and tissues to perform targeted and non-targeted metabolomics studies related to neurological applications. In an ideal scenario, the data and knowledge from both the clinic and basic research is combined in order to gain a comprehensive understanding of disease pathophysiology, thereby facilitating personalized treatment. For the upcoming years, we anticipate a focus on further advancing CE-MS into a robust, quantitative and sensitive approach for metabolomics and bioanalysis studies in general, followed by an increase use of CE-MS in the areas of metabolomics, pharmaceutical analysis, clinical chemistry and biotechnology.