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Next-generation capillary electrophoresis–mass spectrometry approaches in metabolomics

Wei Zhang[1], Thomas Hankemeier[1,2] and Rawi Ramautar[1]

Capillary electrophoresis–mass spectrometry has shown considerable potential for profiling polar ionogenic compounds in metabolomics. Hyphenation of capillary electrophoresis to mass spectrometry is generally performed via a sheath–liquid interface. However, the electrophoretic effluent is significantly diluted in this configuration thereby limiting the utility of this method for highly sensitive metabolomics studies. Moreover, in this set-up the intrinsically low-flow property of capillary electrophoresis is not effectively utilized in combination with electrospray ionization. Here, advancements that significantly improved the performance of capillary electrophoresis–mass spectrometry are considered, with a special emphasis on the sheathless porous tip interface. Attention is also devoted to various technical aspects that still need to be addressed to make capillary electrophoresis–mass spectrometry a robust approach for probing the polar metabolome.

Addresses
[1] Division of Analytical Biosciences, Leiden Academic Centre for Drug Research, Leiden University, The Netherlands
[2] Netherlands Metabolomics Centre, Leiden, The Netherlands

Corresponding author: Ramautar, Rawi (r.ramautar@lacdr.leidenuniv.nl)

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Introduction

The major and ultimate aim of metabolomics is to obtain an answer to a specific biological or clinical question [1]. For this purpose, advanced analytical separation techniques are generally used for the global profiling of endogenous metabolites in biological samples [2••]. Currently, the profiling of endogenous metabolites is commonly performed with mass spectrometry (MS) in combination with an on-line front-end chromatographic separation method [3,4]. Despite significant developments in liquid chromatography column technology and methodology, such as hydrophilic interaction liquid chromatography, the selective and efficient analysis of highly polar and charged metabolites is still highly challenging. Capillary zone electrophoresis, referred to here as CE instead of CZE, separates compounds on the basis of differences in their intrinsic electrophoretic mobility, which is dependent on the charge and size of the analyte, in a capillary filled with separation buffer only under the influence of an electric field. Therefore, CE is highly suited for the analysis of polar ionogenic metabolites. Moreover, as the separation mechanism of CE is fundamentally different from chromatographic-based separation techniques, a complementary view on the composition of metabolites present in a given biological sample is provided. In comparison to chromatographic-based methods the separation efficiency of CE is very high as there is no mass transfer between phases. Actually, under ideal conditions the only source of band broadening in CE is from longitudinal diffusion.

A critical need for metabolomics is also the introduction of analytical methods allowing metabolic profiling of those samples for which the amount is severely limited [5]. CE–MS can be considered an attractive microscale analytical platform for this purpose, as in CE nanoliter injection volumes are employed from (sub-)microliter sample amounts. Therefore, CE–MS is highly suited for the analysis of polar ionogenic metabolites in ultra-small biological samples, as has been recently demonstrated for the analysis of cerebrospinal fluid of mice and extracts from small tissues or a single cell [6–8].

At present, the use of CE–MS for metabolomics studies is disproportionately low in comparison to other analytical separation techniques [2••]. The scientific community still perceives CE–MS as a technically challenging approach suffering from a relatively poor reproducibility and sensitivity [9]. An important reason for this perception is lack of expertise with this technology. In this context, it is of interest to note that CE–MS has been used for the global profiling of native peptides and endogenous metabolites in a clinical context for more than a decade now [10•,11–13]. For example, Mischak and co-workers have analyzed peptides in more than 20 000 human urine samples at different laboratories with an acceptable inter-laboratory reproducibility [10•,14].

Over the past few years, various novel CE–MS approaches have been developed which show a strong potential for improving the sensitivity/metabolic coverage and sample throughput in metabolomics. In this paper, attention will be paid to advancements that significantly improved the analytical performance, particularly with regard to improving the metabolic coverage, of CE–MS for metabolomics studies. Analytical aspects that still need to be addressed

to make CE–MS a viable approach in the metabolomics field are also discussed. Strategies to improve the stability of CE–MS in terms of migration times, data pre-processing aspects, procedures for the identification of metabolites and preconcentration techniques to improve the loadability of CE are not covered in this paper. The reader is referred to more dedicated literature for an overview concerning these topics [15–19,20•,21].

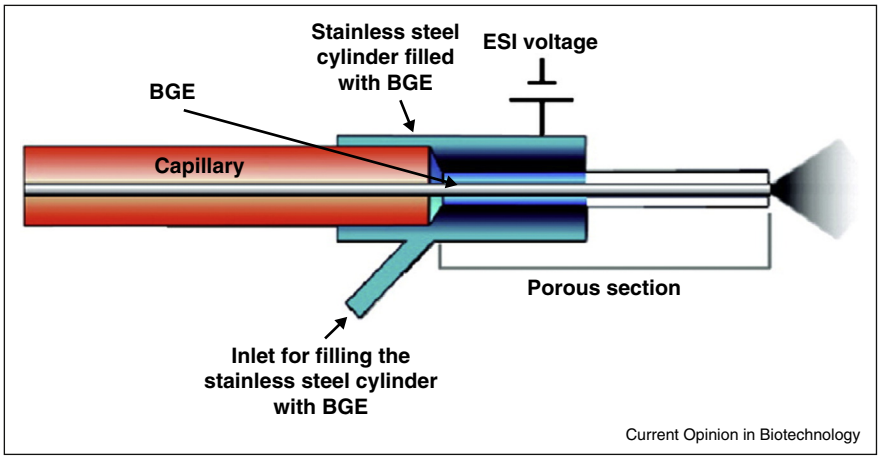
Interfacing techniques for seamless integration of CE and ESI–MS

CE is fundamentally a low flow nanoscale separation technique reaching its optimal separation performance at very low flow-rates, which is typically in the range of 20–100 nL/min depending on the pH of the separation buffer when using a bare fused-silica capillary. Actually, a high separation resolution is obtained in CE by solely separating the compounds on the basis of their electrophoretic mobilities, that is, under (near-)zero electro-osmotic flow conditions. The intrinsically low flow-rates of CE are also advantageous from a viewpoint of the ESI mechanism. In ESI, smaller droplets are generated under low-flow separation conditions, which results in a more efficient desolvation and an improved transfer of ions to the MS [22–24]. Moreover, at very low flow-rates ($\leq$20 nL/min) ion suppression is significantly reduced resulting in an improved concentration sensitivity [22], which is important for in-depth metabolic profiling studies.

In a standard CE set-up both ends of the separation capillary are immersed in buffer vials to which electrodes are added to provide a high voltage gradient. To couple CE to MS, the outlet vial must be replaced by an interface to close the electrical circuit and which provides contact with the ESI stream. Therefore, a CE–MS interface needs to apply voltage to the capillary outlet while maintaining independent CE and ESI electrical circuits. A co-axial solvent delivery as a terminal electrolyte reservoir (i.e., a sheath-liquid interface) and various other interfacing techniques have been subsequently developed to enable the hyphenation of CE to MS. So far, most CE–MS-based metabolomics studies have been performed with a sheath–liquid interface [13,25–30]. CE–MS approaches utilizing a sheath–liquid interface for global metabolic profiling studies were first developed by Soga and co-workers [12,31]. The sheath–liquid interface, originally developed by Smith and co-workers [32], has been used for a broad range of bio-analytical applications with acceptable analytical figures of merit. However, the sheath–liquid is generally provided at a flow-rate between 5 and 10 μL/min, thereby significantly diluting the CE effluent resulting in compromised detection sensitivities for metabolomics applications. Still, an important advantage of the sheath–liquid interface is that the composition of the sheath–liquid can be tuned to modify the ionization efficiency without affecting CE selectivity and efficiency. For example, to improve the detection sensitivity, supplementation of the sheath–liquid with modifiers has been investigated [33]. Enhanced supercharging of analytes in ESI–MS has also been explored by adding various supercharging agents to the sheath–liquid [34]. The effect of these agents on metabolic profiling studies by CE–MS needs to be studied. Overall, considering the fact that both CE and ESI–MS perform most optimally at low flow-rate conditions, the coupling of CE to MS should preferably be carried out via an interfacing technique which effectively utilizes the inherently low flow separation property of CE and the improved ESI efficiency under these conditions.

Currently, the design of new interfacing techniques for CE–MS and assessing their potential for proteomics and metabolomics studies is an active area of research [35–41]. New methods that abolish or minimize the usage of a

Figure 1



Design of the sheathless porous tip interface. A scheme of the porous tip interface, originally developed by Moini, is depicted. *Source*: Reproduced from [43] with permission.