Short Communication

Two capillary approach for a multifunctional nanoflow sheath liquid interface for capillary electrophoresis-mass spectrometry

CE hyphenated to ESI-MS (CE-ESI-MS) is a well-established technique to analyze charged analytes in complex samples. Although various interfaces for CE-MS coupling are commercially available, the development of alternatives which combine sensitivity, simplicity, and robustness remains a topic of research. In this work, a nanoflow sheath liquid CE-MS interface with two movable capillaries inside a glass emitter is described. The setup enables a separation mode and a conditioning mode to guide the separation capillary effluent either into the electrospray or to the waste, respectively. This enables to exclude parts of the analysis from MS detection and unwanted matrix components reaching the mass spectrometer, comparable to divert valves in LC-MS coupling. Also, this function improves the overall robustness of the system by reduction of particles blocking the emitter. Pre-conditioning with electrospray interfering substances and even the application of coating materials for every analysis is enabled, even while the separation capillary is built into the interface with running electrospray. The functionality is demonstrated by analyses of heavy matrix bioreactor samples. Overall, this innovation offers a more convenient installation of the interface, improved handling with an extended lifetime of the emitter tips and additional functions compared to previous approaches, while keeping the higher sensitivity of nanoflow CE-MS-coupling.

Keywords:
Capillary Electrophoresis / CE-MS / Interfacing / Mass Spectrometry

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CE coupled to MS (CE-MS) offers great separation of charged analytes, however, a major disadvantage of standard electrospray (ES) interfacing is the low concentration sensitivity, mainly due to the high sheath liquid flow rate in contrast to the small peak volumes. In recent years, novel designs focused in miniaturization with micrometer emitter tips to enable flow rates in the nanospray regime and match the dimensions of the separation system, as summarized in a recent review article [1]. By these means, remarkable improvements in sensitivity from 10 to 100 times in comparison to conventional interfacing were reported [2, 3]. While these improvements in sensitivity are beneficial, the ease of use and handling might be limited because of fragile parts and small dimensions. Sheathless interfaces are straight forward as a matter of principle as they avoid the use of a sheath liquid by spraying the background electrolyte only, and therefore, avoiding dilution completely. Unfortunately, the establishment of a stable electrical contact for grounding of the separation current and application of the electrospray voltage is challenging but was encountered by various technical solutions such as miniature cracks in the capillary wall, conductive coatings, or a porous tip for the exchange of charge [1, 4]. However, manufacturing precision is crucial in some of these approaches and make commercial spare parts cost intensive which might be detrimental for a widespread utilization of these interfaces, also because the use of custom-made capillaries is problematic for users outside of academia. The direct spray of the background electrolyte compromises separation and ES and also limits postseparation modification, but offers high sensitivity which was demonstrated in many applications and comparative studies [5–7]. In contrast, nanoflow sheath liquid interfaces enable the decoupling from separation and ES conditions by using a make-up liquid for current dissipation and application of the ES voltage, but reducing the dilution to a minimum [8, 9]. These interfaces require an additional emitter that sprays the combined separation capillary effluent and sheath liquid for ES modification. In many cases, nanoflow sheath liquid interfaces are more versatile in use of various capillary types and allow separations without flow in general, which is limited in sheathless approaches. In the here presented system, micropipettes made of borosilicate glass are used due to production at low costs,
optical transparency, and good reproducibility in manufacturing. Interfaces in a similar arrangement, using this kind of emitters, were already applied in various applications of research groups demonstrating high sensitivity [10, 11]. These interfaces were improved over the course of several generations [12] and even commercialized (EMASS-II, CMP Scientific, Brooklyn, NY). In conjunction with the interfaces using borosilicate glass emitters, the terms “electrokinetically pumped” or “EOF driven” sheath liquid interface were introduced and widely spread by the research group of Dovichi and co-workers [13], but are misleading in explaining the actual driving force of the sheath liquid flow. A detailed characterization of this interface demonstrated that the EOF has, if at all, only a minor influence on the flow rate and no influence on the overall flow direction of the sheath liquid [2]. In both negative and positive mode, the sheath liquid flow rates were experimentally determined and generally increase with the applied ES voltage independent of the polarity. Nevertheless, a publication by the Dovichi group [14] suggests an internal emitter coating to reverse the sheath liquid EOF in negative mode and enable a stable spray. Contradicting results were published [2, 15], demonstrating excellent limits of quantification and reproducibility of anions, such as halogenated acetic and sulfonic acids, in drinking water analysis, using uncoated emitters and negative MS detection without difficulties described by Sarver et al. All these results provide strong evidence that the main driving force is not the EOF inside the emitter but rather simply the consumption of liquid by the ES and its substitution by liquid from a reservoir.

However, despite the significant gain in sensitivity, the practical use is still impaired due to technical difficulties in assembling the interface, blocked emitter tips by particles and limited flushing routines of background electrolyte and sheath liquid. Also, manual replenishment of sheath liquid can be tedious and lead to uncontrolled electrolyte and sheath liquid compositions in the emitter tip by accident, as described in a recent publication [16]. These drawbacks prevent routine application of these nanointerfaces. The here presented setup (Fig. 1) demonstrates a technical solution to encounter these issues which allows an easier handling and reduced failure of the emitters, thus, an improved overall robustness. Apart from additional functionalities, such as advanced rinsing protocols with running ES, the liquid composition in the emitter tip can be contained while flushing the capillary with high flow rates. The functional principle of a nanoflow sheath liquid interface with two movable capillaries is presented and the benefits discussed in detail. An example for a technical realization is shown and applied for various types of samples in coupling to mass spectrometers from various vendors (Agilent, Bruker, Thermo).
In the interface setup presented in Fig. 1, two capillaries (365 μm OD each) are guided in parallel through a PEEK tube (10 mm length, 1.0 mm ID) into a PEEK cross union (1.0 mm ID) into a borosilicate ES emitter with orifice openings in the micrometer range (5.5 cm length, 1.0 mm OD and 0.78 mm ID, 15 or 30 μm tip ID, Gynemed, Germany). The outer diameter of the separation capillary (e.g., 30 or 50 μm ID) is reduced on the outlet side, either by etching with hydrofluoric acid or grinding into a cone shape [17]. However, the precision range for reduction of the outer diameter the capillary outlet and capillary positioning are not as important as in sheathless approaches, as demonstrated in previous work [2].

The second capillary has a larger internal diameter (100 μm ID) and a blunt tip to deliver sheath liquid, continuously supplied by a syringe pump. Both capillaries can be moved in axial position. In the backside, the emitter is open to ambient pressure via the third port of the cross union which drains the excess liquid. Therefore, the emitter is free of pressure and the ES consumption is unaffected by the pumping flow rate. The remaining port is used to ground the separation current and to apply the ES voltage with a platinum electrode, either connected to the ground or mass spectrometers power supply. Positioning of the cross union in front of the MS entrance is performed by a XYZ-stage. A digital microscope is used to adjust the emitter tip position relative to the mass spectrometer inlet and position the capillaries inside the emitter.

To prevent the capillaries from protruding and breaking the emitter tip by sliding too far into the frontal position, mechanical stops are attached to the capillaries which allow a reproducible positioning in the micrometer range. The sheath liquid capillary has a length of roughly 20 cm and is connected to the syringe pump by a PEEK tubing with 1 mm internal diameter to reduce backpressure and allow the use of a 10 μm inline filter (Upchurch Scientific) to prevent particles entering.

For initial assembly, the cross union is attached to the XYZ stage and both capillaries are inserted. The electrode and tube for draining the excess sheath liquid are then connected and the syringe pump is switched on. Finally, the emitter is slid on both capillaries and connected to the cross union. Air and particles introduced by the assembly are flushed out of the draining port by the sheath liquid. During this process, the capillaries can be positioned with help of the digital microscope and the mechanical stops adjusted.

Switching of capillaries allows the interface to function as a kind of valve which offers several advantages over traditional approaches. In one capillary, the separation is performed while the other delivers a sheath liquid in a continuous flow. In practice, they can be switched between two positions. The two positions are corresponding to conditioning mode and separation mode (Fig. 1A and B). This allows to control the constitution of liquid in the very tip of the emitter and, therefore, acts as a valve, used to decide whether the separation capillary effluent reaches the mass spectrometer or not. Analysis is performed in separation mode so that migrating analytes exit the capillary outlet and mix with the sheath liquid to be sprayed. In-between analyses, the separation capillary is drawn behind the sheath liquid capillary so that the capillary positions are switched (conditioning mode) and the effluent is flushed out backwards by the continuous flow of sheath liquid. The possibility to control the flow direction of the separation capillary effluent offers a multitude of advantages, such as capillary conditioning with non-ESI compatible agents, application and refreshing of capillary coatings, reduced plugging of the emitter by particles, lower MS contamination and overall improved handling. These benefits are described in the following:

Obviously, fused silica capillaries require initial conditioning with ES incompatible substances, such as hydrochloric acid and sodium hydroxide, for activation of silanol groups and rinsing steps between analyses to re-establish initial conditions. For all other CE-MS interfaces, the capillary needs to be removed from the sprayer to prevent contamination of the ES source and mass spectrometer. In the here presented setup, the initial conditioning can be performed with a built-in capillary in conditioning mode (Fig. 1A and B) without contamination of the mass spectrometer, even with running ES. By these means, also in-between analysis the capillary can be rinsed with any type of cleaning agent for regeneration. This is an important benefit, especially when sample constituents are binding to the capillary wall, leading to systematic migration time shifts. Likewise, coating agents can be applied initially with built in separation capillary and a recoating can be performed before every run. This was successfully performed with successive multilayer coatings for EOF reversal and semipermanent neutral coating materials to suppress the EOF. The ability to restrict the separation capillary effluent from flowing into the emitter tip also reduces blocking of the needle. This is, because in conditioning mode particles and sample residues are flushed out to the backside of the emitter which otherwise could potentially block the micrometer opening of the emitter tip. In fact, this was a serious issue in previous setups which used micrometer orifice needles with only one fixed capillary where all liquid from replenishment had to exit the emitter orifice, increasing the risk of blockage. This issue was also described by Dovichi and Co-workers [12], whereupon 30 μm orifice diameters were preferred over smaller diameters in the third generation. The possibility to divert the capillary effluent in the two capillary approach minimizes the risk of blockage, which turned out to be especially handy for the nontargeted analysis of bioreactor substrate samples, consisting of decomposed animal manure and plant matter from an anaerobic digestion (Fig. 2A1). It allowed a direct injection of the samples with a filtration step as only sample preparation, whereupon several thousand molecular features could be detected. Transient isotachophoresis enabled a large volume injection with sharp peaks, however, because of the high salt concentration in the sample, mass spectra of minutes 5–22 show a continuous background signal without distinct signals. Figure 2B1 shows a replicate analysis of A1, but the exclusion of these high abundant and fast migrating matrix components in conditioning mode till minute 21. Therefore, the interface enables the exclusion of matrix components from the analysis from reaching the mass...
Figure 2. (A1) Base peak electropherogram of a bioreactor sample with heavy matrix after filtration (0.2 μm) and adjustment to pH 2 in a 10% acetic acid separation electrolyte in a 90 cm × 50 μm fused silica capillary with +30 kV separation voltage. MS spectra of minutes 5 to around 22 show a continuous background signal with no distinct peaks, probably consisting of fast migrating salts. (A2) Faster migrating zone was excluded by diverting capillary effluent to waste in conditioning mode from 0 to 21 min (Compare Fig. 1A). (B1 and B2) show microscopic images of a blocked emitter situation if capillary was preconditioned in separation mode (capillary outlet is etched to 20 μm wall thickness). Precipitated particles which remained in capillary during separation are flushed out and accumulate in emitter tip. This case leads to a blocked orifice and exchange of emitter is mandatory. (C1–C4) show a series in conditioning mode while preconditioning (separation capillary concealed) to prevent blocking of emitter tip with matrix residues. Particles are transported to waste by sheath liquid during flushing of background electrolyte.

The presented setup with two movable capillaries offers the possibility to guide the separation capillary effluent away from the emitter tip and prevent unwanted substances from entering the mass spectrometer. Intensive rinsing procedures with ES incompatible cleaning agents and the application of coating materials of built-in capillaries even with running ES are possible, which improves robustness and allows more flexibility for the user. Also, the emitter lifetime is strongly improved over previous setups since particles are guided out to the backside of the emitter. Therefore, smaller emitter orifice diameters could be reconsidered in future experiments to achieve an even more sensitive ES. These advancements over previous setups facilitate the next steps in the evolution of CE-MS interfacing.

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