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A robust and extendable sheath flow interface with minimal dead volume for coupling CE with ESI-MS

Pan Fang, Jian-Zhang Pan, Qun Fang*

Institute of Microanalytical Systems, Department of Chemistry, Zhejiang University, Hangzhou 310058, China

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ABSTRACT

In this paper, we describe a robust sheath flow-based CE-MS interface with minimal interface dead volume based on an extended pattern. A 20 μm i.d. × 90 μm o.d. fused-silica capillary with a chemically-etched thin-wall tip (30 μm o.d.) was used as the separation capillary as well as electrospray emitter, and a 200 μm i.d. × 375 μm o.d. capillary with a tapered tip (40 μm o.d.) was used as the sheath flow capillary. An extendable sheath-flow interface mode was adopted by decreasing the thickness of separation capillary tip and extending the separation capillary tip out from the sheath flow capillary tip, and allowing the sheath flow to be transferred to the separation capillary tip along its outer surface, forming a surface sheath flow to mix with sample flow at the separation capillary tip. Such a strategy could significantly reduce the interface dead volume and thus improve the CE separation efficiency and detection sensitivity, as well as evidently enhance the working reliability of the CE-MS interface. We investigated various factors affecting the interface performance, including capillary extending distance, emitter diameters, sheath flow capillary shape, and sheath flow rate. Under the optimized conditions, a minimal interface dead volume of ca. 4 pL was obtained which is the smallest one compared with previously-reported sheath flow-based CE-MS interfaces. The feasibility and applicability of the present CE-MS interface were demonstrated in the separation of a peptide mixture with high separation efficiency of 2.07–3.38 μm plate heights and good repeatabilities (< 6.1% RSD, n = 5). We except such a simple and robust interface could provide a possible solution for the development of commercial CE-MS interfaces differing from the currently-used ones, and has the potentials to be applied in routine analytical laboratories for various studies such as proteomics, metabolomics, or single cell analysis.

1. Introduction

Capillary electrophoresis-mass spectrometry (CE-MS) is a powerful tool for the analysis of complex polar compounds and charged compounds such as metabolites [1,2], intermediates [3], neurotransmitter [4], nucleotides [5], peptides and post-translationally modified proteins [6], which provides an effective complementary to liquid chromatography-mass spectrometry (LC-MS) technology. Currently, electrospray ionization technology is widely used in achieving the combination of CE and MS, due to its simple structure and operation, good on-line associativity and multiple ionization modes for positive and negative particles. In most of reported CE-ESI-MS systems, interfacing modes of sheathless and sheath-flow [7–13] are mainly used to couple a CE system with an ESI-MS system.

The sheathless interfaces commonly use the separation capillary as the electrospray emitter, in which a stable and durable electrical contact is required to be established at the electrospray emitter tip end and some volatile separation buffers need to be used. Moini et al. [9] developed a sheathless interface for CE-ESI-MS using a porous tip fabricated by etching with 49% HF solution to achieve electrical connection. This interface was applied to CE-MS analysis of amino acids, proteins and protein digestion products. The sheathless interfaces can eliminate sample dilution in the interface and are relatively easy to build. However, the micropores in this type of interfaces are easily blocked by impurities or capillary coatings, which may limit their application in complex samples. For the CE-ESI-MS interfaces under the sheath-flow mode, Chen's group proposed a solution using a custom-designed stainless steel hollow needle surrounding the separation capillary terminus [10]. The steel needle with a tapered tip (75 μm cross-section i.d.) acted as an electrode for CE outlet and an electrospray emitter for MS. This interface was applied in the separation of cytochrome C, lysozyme and ribonuclease A with a neutral capillary and

Abbreviations: LIF, laser-induced fluorescence; FITC, fluorescein isothiocyanate; CE, capillary electrophoresis; POCT, point of care testing; NA, numerical aperture; LED, light-emitting diode; PD, photodiode; APD, avalanche photodiode; ADC, analog-to-digital converter; SNR, signal to noise ratio; FSC, forward scattered

* Corresponding author.
E-mail address: fangqun@zju.edu.cn (Q. Fang).

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angiotensin I and II with a polyethyleneimine-coated capillary. To simplify the interface fabrication, Dovichi's group developed an electrokinetically pumped sheath flow interface for high efficient and sensitive peptide analysis [11]. In this design, the separation capillary was inserted into a glass electrospray emitter using a cross fitting. The authors demonstrated that minimizing the distance between the capillary exit and the emitter tip benefited for improving peak height, peak width and asymmetry. The best results were observed with a 2 μm diameter emitter tip and 1 mm spacing between the separation capillary tip and the emitter tip. The separation efficiency exceeded 200,000 plates for insulin receptor. Since decreasing the capillary-to-tip distance was advantageous, in the subsequent work of this group, they etched the outer wall of the separation capillary from a diameter of 150 μm to ~ 60 μm to install the capillary end closer to the emitter orifice [12]. In their second generation interface, the optimum distance was ca. 200 μm, the emitter tip diameter was 8 μm and the sensitivity was increased for measurement of *E. coli* digestion product. However, due to the narrow size of 2–10 μm of the emitter orifices, the tip blocking problem may occur frequently and thus shorten the working lifetime of the interface. Therefore, they reported the third-generation interface employing a larger diameter emitter orifice with 15–35 μm tips and the capillary-to-tip distance was decreased to 20 μm [13]. It was illustrated that the loss in sensitivity associated with the larger orifice size with an estimated dead volume ca. 30 pL was able to be recovered by decreasing the capillary-to-tip distance. Recently, Gonzalez-Ruiz et al. proposed a straight interface design by passing a separation capillary through a blunt stainless steel needle with a reducing sleeve and a tee [14]. A sheath liquid capillary connected to the orthogonal inlet of the tee by a nanoflow pump. The dimensions of the separation capillary and blunt needle were 50 μm i.d., 323 μm o.d. and 408 μm i.d., 718 μm o.d., respectively, and the capillary protruded 200 μm out from the needle. The authors illustrated two types of Taylor cones with the base diameters equal to the outer diameters of the needle and the capillary tip, corresponding to estimated Taylor cone volumes of ca. 125 nL and 7 nL, respectively. The authors also applied the interface in the analysis of basic drugs by CE-TOF/MS with smaller peak widths of 7–13 s (full width at half maximum) compared with conventional interfaces with peak widths of 9–16 s.

Since 2015, the authors' group has conducted the work on coupling CE with MS using the strategy of extending separation capillary from sheath flow capillary to obtain minimal interface dead volume and improve the interface robustness. The separation capillary was used as electrospray emitter, and sheath flow that delivered out from a sheath flow capillary sleeved the separation capillary was transferred to the separation capillary tip along its outer surface. With such a strategy, the size of the Taylor cone at the capillary tip is mainly determined by the capillary tip diameter, thus we used chemical etching method to reduce the wall thickness as thin as possible. We observed that the tip diameter of the sheath flow capillary also had significant effects on reduction the dead volume, and was required to be optimized to ensure stable and continuous Taylor cone. Various factors affecting the interface performance, including capillary extending distance, emitter diameters, sheath flow velocity and sheath flow matrix effect, have been investigated. Under the optimized conditions, a minimal Taylor cone with a volume of ca. 4 pL was obtained using a separation capillary with 20 μm inner diameter and 30 μm tip outer diameter. To demonstrate the feasibility of the present CE-MS interface, we applied it in the separation a mixture of four peptides and high separation efficiency of 2.07–3.38 μm plate heights and good repeatabilities (< 6.1% RSD, n = 5) were obtained.

2. Material and methods

2.1. Chemicals and reagents

Standards of kemptide, leucine encephalin, bradykinin and angiotensin I were purchased from GL Biochem Co. (Shanghai, China). Reserpine and rhodamine 110 were purchased from Aladdin (Shanghai, China). Ammonium acetate, formic acid, acetic acid, and methanol were from Sigma-Aldrich (St. Louis, USA). Hydrofluoric acid was from Sinopharm Chemical Reagent Co. (Shanghai, China). Teflon AF was purchased from Dupont (Teflon AF 1600, Wilmington, USA). All the aqueous solutions were prepared with deionized water.

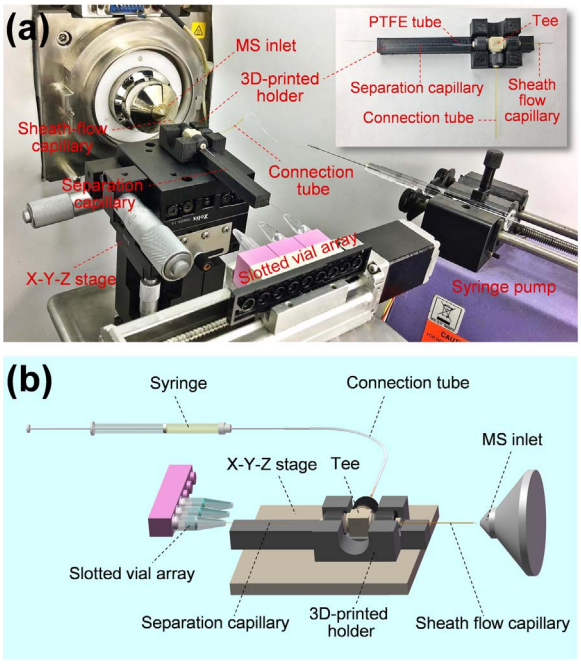

Fig. 1. Images (a) and schematic diagram (b) of the present CE-ESI-MS system with the extendable sheath-flow interface. The inset in (a) shows the image of the whole interface.

2.2. Extendable sheath-flow CE-ESI-MS interface

The image and schematic diagram of the extendable sheath-flow CE-ESI-MS interface are shown in Fig. 1. The interface employed coaxial sheath flow pattern with two fused silica capillaries. A 15-cm-long capillary (20 μm i.d., 90 μm o.d., 12 μm thickness polyimide coating, Polymicro Technology Inc., Phoenix, USA) was used as the separation capillary. The outlet end of the separation capillary was treated with a hydrofluoric acid solution to obtain a tapered tip by first removing 5-mm polyimide coating at the capillary end, blocking the capillary channel at its the other end with paraffin to prevent etching of the inner capillary wall, and then inserting the exposed capillary part into a 40% hydrofluoric acid solution for 22 min for etching the outer capillary wall. After removing the paraffin in the capillary and being washed with 1 M NaOH solution and water for 5 min, a tapered tip with an outer diameter of ca. 30 μm and uniform inner diameter of 20 μm was formed at the capillary outlet end served as the ESI emitter. A thicker capillary (200 μm i.d., 375 μm o.d., Reafine Chromatography Co., Yongnian, China) with a conical tip with ca. 35 μm inner diameter and 40 μm outer diameter was used as the sheath flow capillary. The conical tip of the sheath flow capillary was fabricated by using pulling method with a butane flame [15]. A hydrophobic treatment with Teflon AF was performed to the outer surface of the sheath flow capillary to avoid the sheath flow from overflowing along the capillary outer surface. First, the sheath flow capillary was washed with 1 M NaOH solution and water for 10 min. After drying with nitrogen, the capillary tip was immersed into a 0.6% (w/v) solution of Teflon AF in fluorinert FC-40, pulled out slowly and then dried naturally for 1 min. Finally, the capillary was heated to 165 °C for 5 min, 330 °C for 15 min and allowed to cool to room temperature in a muffle furnace (Sigma Instrument