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RESEARCH ARTICLE

Portable, Battery Operated Capillary Electrophoresis with Optical Isomer Resolution Integrated with Ionization Source for Mass Spectrometry

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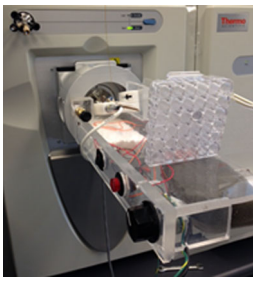

Abstract. We introduce a battery operated capillary electrophoresis electrospray ionization (CE/ESI) source for mass spectrometry with optical isomer separation capability. The source fits in front of low or high resolution mass spectrometers similar to a nanospray source with about the same weight and size. The source has two high voltage power supplies (±25 kV HVPS) capable of operating in forward or reverse polarity modes and powered by a 12 V rechargeable lithium ion battery with operation time of ~10 h. In ultrafast CE mode, in which short narrow capillaries (≤15 μm i.d., 15–25 cm long) and field gradients ≥1000 V/cm are used, peak widths at the base are <1 s wide. Under these conditions, the source provides high resolution separation, including optical isomer resolution in ~1 min. Using a low resolution mass spectrometer (LTQ Velos) with a scan time of 0.07 s/scan, baseline separation of amino acids and their optical isomers were achieved in ~1 min. Moreover, bovine serum albumin (BSA) was analyzed in ~1 min with 56% coverage using the data-dependent MS/MS. Using a high resolution mass spectrometer (Thermo Orbitrap Elite) with 15,000 resolution, the fastest scan time achieved was 0.15 s, which was adequate for CE-MS analysis when optical isomer separation is not required or when the optical isomers were well separated. Figures of merit including a detection limit of 2 fmol and linear dynamic range of two orders of magnitude were achieved for amino acids.

Keywords: Battery operated CE, Ultrafast CE, Integrated ionization source, Optical isomers, Amino acids

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Introduction

In the past several years, there has been considerable interest in developing handheld devices for the detection of chemicals and biological samples [1, 2]. For example, handheld Raman has been used for the detection of organic compounds such as controlled substances [1, 2]; however, since the detection is based on spectroscopic techniques, the results are preliminary and good for screening purposes, and the findings still need to be analyzed by confirmatory laboratory techniques such as separation followed by mass spectrometry. To address this need, several handheld and field portable mass spectrometers with MS/MS capability and fast data acquisition rates have been developed and applied to the detection of several classes of chemical and biological compounds in the fields of forensics, food industry, industrial hygiene, quality, and consumable products [3–6] with a high degree of specificity. To provide real-time analysis, several ionization sources, including desorption electrospray ionization (DESI), direct analysis in real time (DART), and paperspray ionization have been developed and used in conjunction with handheld, field portable, and benchtop mass spectrometers [7–11]. These ionization sources lack or have limited separation capability. To add separation capability while maintaining speed of analysis, ion mobility has been added [12]; however, because separation occurs after ionization, ion suppression during the ionization of dirty samples can still affect the analysis. Also, dilution and infusion of samples with suspected suppressants may render the solution too dilute for MS analysis. In addition to separating salts, cutting agents, or other impurities, which can suppress or interfere with the detection of analytes of interests, the use of separation enhances specificity by adding another layer of compound identification (retention or migration time) to the analysis. Moreover, separation improves quantitation by providing peak integration capability. Yet another advantage of the use of separation techniques is their capability for on-line sample concentration [13]. A variety of fast separation

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techniques, such as fast GC and fast LC, as well as supercritical fluid chromatography MS and capillary electrophoresis MS (CE-MS), have been introduced [13–17]. Among these separation techniques, CE is the easiest one to miniaturize since it only requires high voltage power supplies for its operation. To provide fast analysis time, usually the capillary length is reduced and electric field strength is increased. To minimize heat generation, narrower channels are used. Miniaturized CE has been introduced in both capillary and chip formats [18–21]. While the chip format is more versatile by including a variety of functions on a chip, by using cylindrical and narrower channels that provide more efficient heat dissipation, the capillary format can provide higher separation efficiency [22]. A combination of chip (for sample preparation and injection) and capillary (for separation) formats has also been introduced, but this format is still not available for high electric field strengths (≥1000 V/cm) [23]. High resolution separation achieved under high electric field strength is especially useful in cases where optical isomer separation is essential for compound identification, such as for detection of several classes of illicit drugs, where sentencing may depend on the optical isomer, in neurobiology, where d-isomers are implicated as neurotransmitters, in pharmaceutical/food science, where more than half of the drugs currently in use are chiral compounds [24], and in astrobiology, where identification of stereoisomers of amino acids has profound effects on the issue of life on other planets [25]. In this manuscript, we introduce a battery powered CE/ESI source, which includes all the necessary power supplies and an integrated electrospray ionization tip in one portable package. The portable CE/ESI ionization source fits in front of mass spectrometers, similar to any nanospray ion source; however, it provides high separation efficiency, including optical isomer separation in about 1 min when it is used under ultrafast CE mode.

Experimental

Battery Operated CE/ESI Ionization Source

Figure 1 shows the picture of the portable CE/ESI ion source (left) and its schematic (right). The source housing is constructed using Plexiglas and includes two high voltage power supplies (HVPS-EMCO, Sutter Creek, CA, USA) with output voltages of up to either 25 kV or –25 kV. The output voltage of the HVPSs can be adjusted using a potentiometer in the range of ±4 kV to ±25 kV (Figure 1). A switch on the CE/ESI ion source is used to switch between positive or negative voltage. The HVPSs are powered by a rechargeable 12 V lithium ion battery (9800 mAh). Since the output current of the narrow CE capillaries used in this study is in the low µA, one charge of the battery usually lasts for the entire day (~10 h of operation) without any recharging. After about 5 h of use at –25 kV, the battery was still 50%–75% charged, as indicated by the LED lights of the battery. The outputs of the HVPSs are attached to the CE inlet electrode, which is used for injections and separation. The whole system, including the high voltage power supplies and the rechargeable battery, is less than 4 pounds. The "ground" wire of the portable CE/ESI ion source is connected to the mass spectrometer chassis ground. The ESI voltage, which is provided by the mass spectrometer, acts as the

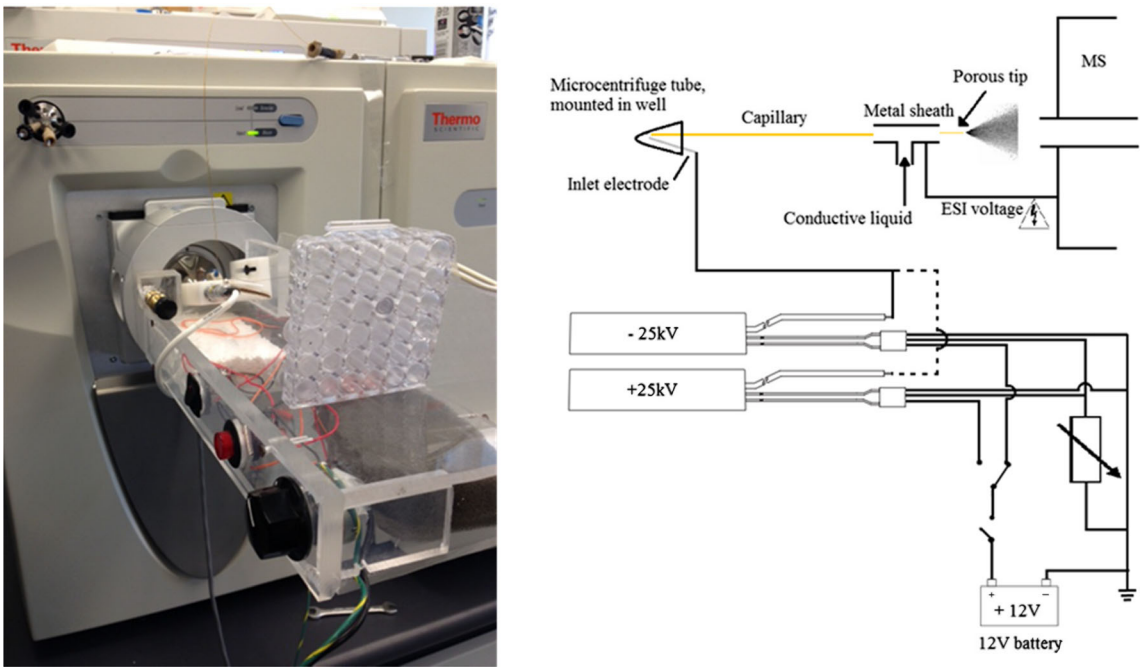

Figure 1. Left: picture of the CE/ESI source mounted on the Thermo IonMax source adaptor. Right: schematics of the source

CE outlet/ESI electrode. Samples and background electrolytes (BGEs) are placed in a 96-well plate (Figure 1). The Thermo nanospray Ion MAX adaptor was used to attach the CE/ESI source to the mass spectrometer. The bottom Plexiglas of the source is constructed to be identical to the bottom of a Thermo nanospray Ion MAX base mount so that it could be easily exchanged with the Ion MAX nanospray ionization source. A short (20–25 cm) capillary with a porous tip was used for the separation and ionization [17, 26]. The capillary was passed through the metal sheath (Figure 1) filled with a conductive solution containing 0.1% formic acid in water and protruding from the sheath outlet by ~1 cm. The outlet tip of the capillary was positioned at ~1 mm from the inlet of the mass spectrometer for maximum sensitivity.

Sample Preparation

All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA) unless otherwise specified. An amino acid standard mixture was used as is or diluted using the BGE to desired concentrations. For the limit of detection and linearity study, 1 mg/mL solutions of L-aspartic acid (L-Asp), L-isoleucine (L-Ile), L-phenylalanine (L-Phe), L-asparagine (L-Asn), and L-glutamine (L-Gln) were mixed and diluted to obtain concentrations of 200, 100, 20, 10, 2, and 0.2 µg/mL. Tryptophan was used as an internal standard in these solutions to normalize the peak areas.

Capillary Electrophoresis-Mass Spectrometry (CE-MS) Analysis

Porous tip capillaries (≤25 cm in length) were interfaced with either a Finnigan LCQ Duo (San Jose, CA, USA) or a Thermo Orbitrap Elite (Waltham, MA, USA) mass spectrometer [26]. Various capillary inner diameters (15, 10, and 5 µm) were used to investigate the effect of inner diameter on separation (P/N: 106815-0006, -0004, -0002; Polymicro Technologies, Phoenix, AZ, USA). For CE operation, first the capillary was completely filled with poly-brene to coat the walls to provide electro-osmotic flow towards the outlet under reverse polarity mode (negative HV applied to the inlet electrode) [25]. Then, the capillary was filled with the BGE, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18-C-6-TCA, 97%). Next, samples were injected by placing the capillary inlet and the electrode in the sample well and turning on the voltage (–4 kV) for the desired length of time (1–5 s). The capillary inlet and the electrode were then moved to a methanol well (for cleaning purposes) before they were moved to the BGE well for separation. Separation occurred by applying negative high voltage to the electrode, which separated compounds and provided electroosmotic flow towards the outlet. The electrospray voltage was +1.1 kV, +1.0 kV, and +0.8 kV for 15, 10, and 5 µm i.d. capillary, respectively. When using 5 µm i.d. capillaries, all solutions should be centrifuged and the supernatant should be used to minimize the chances of plugging the capillary. The mass spectrometer heated capillary was 150°C to minimize the fragmentation of 18-C-6-TCA complexes while providing efficient desolvation. The mass spectrometers were scanned in the *m/z* range of 505–650 and 400–1100, respectively, for AA and a tryptic digest of BSA (New England Biolabs, Ipswich, MA, USA). All separations were carried out at –25 kV to achieve the ≥1000 V/cm for rapid separation. To determine the electrical field strength, the voltage difference between the separation voltage and the ESI voltage was divided by the capillary length.

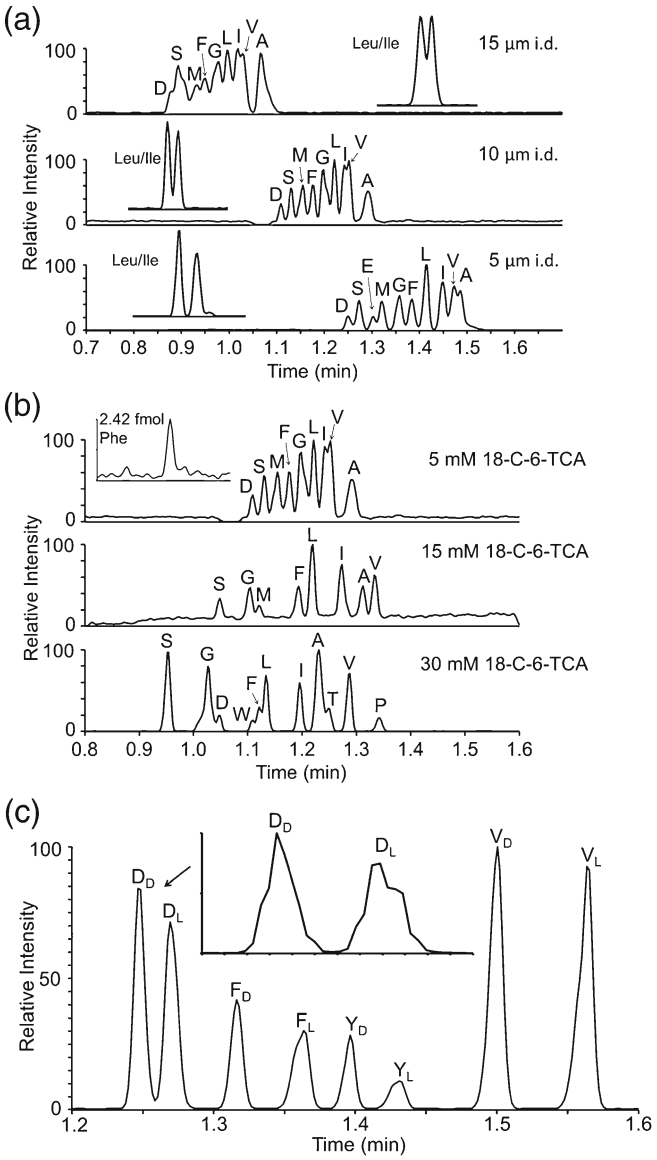

Figure 2. **(a)** Electropherograms of amino acid standard showing improved separation with decreasing capillary inner diameter (insets show separation of Leu and Ile). **(b)** Electropherograms of amino acid standard showing improved separation with increasing 18-C-6-TCA concentration using 10 µm, 25-cm-long capillaries with inset showing 2.42 fmol injection of L-Phe. **(c)** Extracted ion electropherogram showing separation of D and L of four amino acids with inset of extracted ion electropherogram without smoothing showing the number of data points across D- and L-Asp peaks

Table 1. Figures of Merit for Amino Acids

Amino acid	Detection limit (fmol)	Dynamic range (fmol)	Correlation coefficient
L-isoleucine	3.05	9.15–305	0.9700
L-asparagine	15.15	45.45–303	0.9933
L-aspartic acid	3.01	9.03–150	0.9843
L-glutamine	13.70	41.10–274	0.9944
L-phenylalanine	2.42	7.26–242	0.9995

Proteomics Data Analysis

BSA raw file was searched using Sequest in Proteome Discoverer 1.4. Spectra were searched against a UniProt database (07/16/14), with the following search parameters. Error tolerance was set to 20 ppm and 0.6 Da for precursor and fragments, respectively. Static modification (carbamidomethyl of cysteine) anddynamic modifications (oxidation of methionine, deamidation of asparagine and glutamine) were used. All data were filtered using peptide confidence set to "high."

Results and Discussion

Analysis of Small Molecules and Their Optical Isomers by the CE/ESI Ion Source

To investigate the effect of the capillary inner diameter on the separation, the amino acid standard mixture was analyzed using three 25-cm-long capillaries with inner diameters of 5, 10, and 15 µm. Figure 2a shows the effect of inner diameter on separation using 5 mM 18-C-6-TCA as the BGE and a separation voltage of –25 kV. As the inner diameter decreased, the separation of the amino acids increased (Figure 2a). This is evident by looking at leucine (Leu) and Ile where they begin to separate (Figure 2a, insets from top to bottom) with the 15 µm i.d. capillary, they are almost separated (R = 1.1) with the 10 µm i.d. capillary, and they are baseline resolved (R= 1.5) using the 5 µm i.d. capillary. The results demonstrate that the narrower capillaries provide higher electrophoretic resolution. There are several factors that contribute to higher resolution in narrower capillaries. First, narrow capillaries operate at lower currents because of lower charge density in the narrow capillary, thereby reducing joule heating. Joule heating is a major cause of band broadening under the high electric field strength (≥1000 V/cm) used in these experiments [22]. Second, in narrower capillaries diffusion is laterally limited, resulting in the randomization of the analyte ions leading to high resolution [22]. Third, migration times are slightly longer in narrower capillaries, allowing analytes to stay in the capillary for longer time, enhancing resolution (Figure 2a); however, because of the fast analysis time of the UFCE, band broadening due to longitudinal diffusion is minimal.

Previously, we had shown that 18-C-6-TCA is an excellent BGE for separation and high sensitivity of detection for amino acids and their optical isomers using conventional CE-MS [27]. Similar results were obtained using the portable CE/ESI source except that the analysis time was reduced by more than an order of magnitude to ~1 min. A 10-µm i.d., 25-cm-long capillary and separation voltage of –25 kV were used for the analyses shown in Figure 2b. As shown in Figure 2b (from top to bottom), increasing the concentration of 18-C-6-TCA improved the separation of the AAs even further. For example, for Leu and Ile, the resolution increased from 1.1 to 3.7; however, the migration order of some of the amino acids changed at the various concentrations. At 5 mM TCA, Asp migrated first; however, as the concentration of 18-C-6-TCA increased, Ser migrated first. The addition of 18-C-6-TCA to the background electrolyte converts CE to affinity CE, allowing some chromatography to affect the separation.

To investigate the figures of merit of the portable CE/ESI ion source, a mixture of five amino acids was analyzed over a

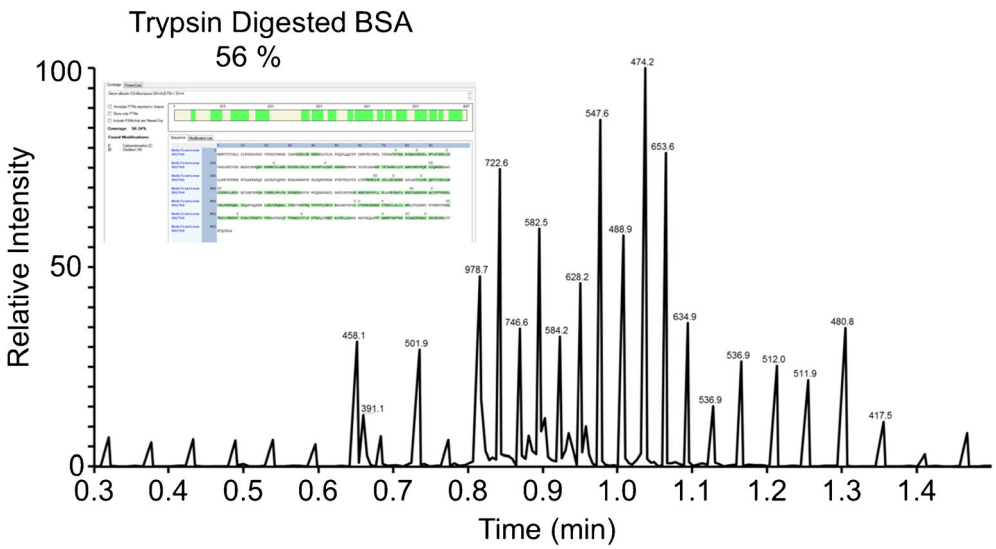

Figure 3. Base peak electropherogram showing CE-MS/MS analysis of ~50 pL of a 1 mg/mL solution of BSA digest with inset showing coverage obtained

concentration range of two orders of magnitude from 2 to 200 µg/mL using 5 mM 18-C-6-TCA as a BGE. Separations occurred at –25 kV using 10-µm i.d., 25-cm-long capillaries. The amino acids analyzed were L-Phe, L-Asp, L-Ile, L-Gln, and L-Asn. Under these experimental conditions, the detection limits for these compounds were 2.42, 3.01, 3.05, 13.70, and 15.15 fmol, respectively. The injection of 2.42 fmol of L-Phe is shown as an inset in Figure 2b. The detection limit, dynamic range, and correlation coefficient for the analysis of these amino acids are shown in Table 1. The calculated approximate injection volume was 0.2 nL based on the migration of neutral amino acids under these conditions. Low injection volume of a narrow capillary is a disadvantage of this technique, especially when used with portable mass spectrometers, which usually have lower sensitivity than benchtop mass spectrometers; however, high sensitivity of the extremely low flow rate of the narrow capillaries in conjunction with the sheathless interface as well as its on-line sample stacking capability partially compensate for this disadvantage [17].

The portable, battery operated CE integrated with ionization source was also efficient at separating the optical isomers of amino acids. Figure 2c shows the baseline separation of four pairs of amino acid D and L enantiomers in ~1.3 min. The inset of Figure 2c shows the expanded migration time axis for D- and L-Asp with smoothing turned off to show the number of data points on each peak of D- and L-Asp. As shown, fast data acquisition is required to obtain baseline resolution for these closely migrating peaks. In low resolution mode, the fastest scan time of the LTQ Velos Pro of the Orbitrap Elite was 0.07 s, which made it compatible with the narrow peaks of UFCE/ESI. Under this condition, baseline separation of amino acids and their optical isomers were achieved in ~1 min. Current portable mass spectrometers are capable of scan speeds >5 scans/s, which would be sufficient for qualitative analysis under these UFCE conditions [28].

Analysis of a BSA Digest Using the CE/ESI Source

In addition to small molecules mentioned above, the portable CE/ESI also performed well in analyzing a complex peptide mixture. For example, by injecting ~50 pL of a 1 mg/mL solution of BSA, ~56% coverage was achieved (see Figure 3). This is a significant improvement in coverage compared with our previous experiment, in which a tryptic digest of yeast enolase was identified with only one peptide [17]. The significant increase in coverage was due to the use of a much faster mass spectrometer in this experiment (LTQ Velos Pro of the Orbitrap Elite with a scan rate of ~0.07s/scan) compared with the mass spectrometer used in the previous experiments (LCQ Classic with a scan rate of ~0.3 s/scan).

Conclusions

The portable, battery operated CE integrated with ionization source is an excellent ionization source for mass spectrometry. It includes all the power supplies needed to operate continuously on battery power for hours. It weighs less than 4 pounds and fits in front of any mass spectrometer similar to an electrospray or a nanospray source; however, it provides high resolution separation of amino acids and their optical isomers in about 1 min. Moreover, it provides excellent detection limits as well as linear dynamic range and reproducibility. In addition, in conjunction with a moderately fast mass spectrometer, it was able to analyze a BSA tryptic digest in about 1 min with ~56% sequence coverage. The portable CE/ESI ion source could be an excellent ion source for an atmospheric pressure sampling portable mass spectrometer.

Acknowledgments

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