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Open-tubular capillary electrochromatography-mass spectrometry with sheathless nanoflow electrospray ionization for analysis of amino acids and peptides

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A novel, rugged sheathless capillary electrochromatography-electrospray ionization (CEC-ESI) device, in which an open-tubular separation capillary and an electrospray tip are integrated with a Nafion tubing junction, is coupled to mass spectrometry (MS) for the analysis of amino acids and peptides. A stable electrospray was generated at nanoflow rates by applying a positive electrical potential at the Nafion membrane junction. To sustain the stable spray, an electroosmotic flow (EOF) to the spray was supported by coating the fused silica capillary with Lupamin, a high-molecular-weight linear positively charged polyvinylamine (PVAm) polymer, which also minimizes analyte adsorption. Electrochromatographic separation of amino acids and peptides was further enhanced by the chromatographic selectivity of Lupamin stationary phase for these molecules. The device was very reliable and reproducible for CEC-ESI-MS analyses of amino acids and peptides for over a hundred injections. The separation and detection behaviors of amino acids and peptides under different conditions including pH, concentration, and composition of mobile phases on Lupamin-coated and uncoated capillaries have been investigated. The relationship between nano electrospray stability and EOF is discussed. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: capillary electrochromatography; electrospray ionization; mass spectrometry; sheathless interface; amino acid and peptide analysis; Lupamin

INTRODUCTION

Capillary electrochromatography (CEC) is a hybrid separation technique that combines the features of both capillary electrophoresis (CE) and capillary high performance liquid chromatography (μ-HPLC). Because the mobile phase is driven by electroosmotic flow (EOF) with a plug-like profile, the dispersions caused by static and flow velocity differences that contribute to the zone broadening in HPLC are minimized. Therefore, CEC can provide high separation efficiency with theoretical plate numbers comparable to those obtained by CE. Additionally, the stationary phase in CEC not only provides specific separation selectivity for analytes but also eliminates the interference on ionization efficiency of additives such as surfactants in micellar electrokinetic chromatography (MEKC) and CE. Mass spectrometry (MS) is one of most useful detection methods for CE and HPLC because it can provide information about the chemical structures and molecular weights of the analytes. In fact, μ-HPLC-MS and CE-MS have become powerful analytical tools in proteomics and metabolomics. As CEC integrates both advantages of high separation efficiency offered by EOF driving mode in CE and high chromatographic separation selectivity offered by stationary phase in μ-HPLC, the hyphenation of CEC with MS represents a very important and promising separation and detection technique, especially for the analysis of saccharides,[1] enantiomers,[2,3] and peptides and proteins.[4]

Electrospray ionization (ESI) as an interface technique for the hyphenation of μ-HPLC, CE, or CEC with MS[5] is ideally suited for generating gas-phase ions from a liquid stream. In capillary electrophoresis/capillary electrochromatography-electrospray ionization-mass spectrometry (CE/CEC-ESI-MS) coupling, a suitable electrical connection between the separation technique and the ESI emitter is a main requirement. Three main interfaces, namely, coaxial sheath-flow interface,[6] liquid junction,[7] and sheathless interface[8] have been used in CE/CEC-ESI-MS. The main advantages of sheath-flow interface are the wide flexibility in selection of separation electrolyte solutions, its reliability, and the existence of several commercial designs that

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make it the most widely used interface in CE-ESI-MS routine analysis. However, owing to the sample dilution and additional background noise from the sheath liquid, the sensitivity in sheath-flow interface decreases in some cases.[9] Liquid junction interface requires tedious capillary alignment and end-to-end butting of the separation capillary and the spray tip. Poor alignment compromises sensitivity and separation efficiency by spreading of the sample in the relatively large dead volume of the liquid junction.[16]

The development of sheathless interfaces attracts much attention because of the compatible flow rates of each technique, which leads to high desorption and ionization efficiency and, consequently, high sensitivity. Several sheathless designs have been reported including (1) the use of the single capillary wherein electrical contact is established by coating the capillary outlet with a conductive metal[8] or polymer[10] or by inserting a conductive wire into the outlet of the capillary[11] or through a small pinhole in the wall of capillary,[12] and (2) the use of two pieces of capillary wherein the CE capillary is connected to a short spray tip via a sleeve. The sleeve includes a piece of microdialysis tubing,[13] stainless steel tubing,[14] a micro-tee,[15] and an etched capillary widow.[16]

Recently, we developed a chip-scale electroosmotic pump/electrospray coupling device using monolithic silica as EOF-generating material and Nafion tubing as the connection of pumping and electrospray capillaries. This device can generate a very stable electrospray with the support of EOF.[17,18] In this paper, we present a novel, sheathless CEC-ESI device coupled to the MS for the separation and detection of amino acids and peptides. For MS detection of such positively charged analytes at acidic experimental conditions, a positive potential must be applied at the spray emitter. A positively polarized emitter (or at the separation/spray junction) would drive the analytes from the spray. Thus, a strong EOF flow must be required not just to pressurize the emitter but also to convect the analytes toward the spray. For the purpose of electrochromatographic separation of amino acids and peptides, chromatographic stationary phase must be immobilized on the inner wall of the separation capillary column. For the current electrochromatographic separation of amino acids and peptides, a new open-tubular capillary column coated with Lupamin, a high-molecular-weight linear polyvinylamine (PVAm) polymer, was used for the first time to satisfy the above complex constraints on the directions and relative magnitudes of EOF as well as chromatographic selectivity for the analytes. The Lupamin-coated column produced the proper EOF direction and magnitude in silica capillaries, and also displayed chromatographic selectivity for amino acids and peptides as an additional advantage. Adsorption of analytes on the capillary inner wall was also suppressed by the Lupamin coating. A stable electrospray was generated at nanoflow rates by applying a potential at the Nafion junction. The device was very rugged and reliable for CEC-ESI-MS analysis of amino acids and peptides for over a hundred injections. Critical effects of separation conditions, such as pH and mobile phase composition, on Lupamin stationary phase stability and the EOF rate and direction on electrospray stability are discussed.

EXPERIMENTAL

Chemicals and materials

Acetic acid and glass slides were obtained from Fisher Scientific (Pittsburgh, PA). Amino acids and peptides were obtained from Sigma-Aldrich (St. Louis, MO). Lupamin 9095 (a high-molecular-weight linear PVAm, average MW 340 000) was obtained from BASF Corporation (Mount Olive, NJ). Nafion tube (i.d. 0.45 mm) was obtained from Perma Pure LLC (Toms River, NJ). Fused-silica capillary with 50 μm i.d. and 365 μm o.d. was purchased from Polymicro Technologies (Phoenix, AZ). Electrospray capillary tip (FS360-50-8) was a sharply tapered capillary obtained from New Objective (Woburn, MA). 4–6 Minute Epoxy adhesive was obtained from Pacer Technology (Rancho Cucamonga, CA). Doubly distilled and deionized (DI) water was used for the preparation of running electrolytes. The sample concentrations were prepared with the running electrolyte in the range of $7.0 \times 10^{-5} - 1.0 \times 10^{-3}$ M. The test sample mixture of amino acids and peptides contains 2.0×10^{-2} M of Leu, 5.4×10^{-4} M Phe, 3.9×10^{-4} M Leu-Gly-Gly, 1.3×10^{-4} M Gly-Leu, and 7.2×10^{-5} M Gly-Phe.

Sheathless nano electrospray interface fabrication

The schematic diagram of experimental setup and sheathless interface is illustrated in Fig. 1. An open-tubular capillary separation column (36 cm) and a sharply tapered spray capillary (4.5 cm in length, tip i.d. 8 μm, o.d. 360 μm, i.d. 50 μm) were butted together inside Nafion tubing (5 mm in length). Epoxy was applied around the outside of

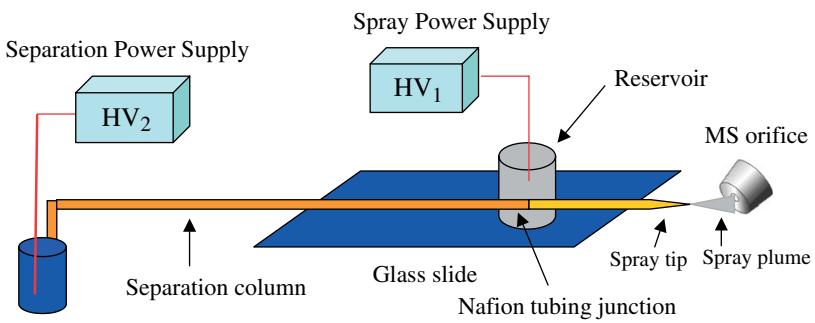

Figure 1. Schematic diagram of CEC-ESI-MS setup and the sheathless interface.

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Nafion tubing/capillary boundaries. After the epoxy dried, the connected capillary was mounted on a glass slide (2 cm × 5 cm). A PVC tube (5 mm i.d., 8 mm in height) was glued on the top of the Nafion junction to form a reservoir. The reservoir was filled with the same running electrolyte as employed in the separation, and electrospray voltage was applied through a platinum wire dipped into the reservoir and connected to the MS power supply (HV_1). The use of an open reservoir rather than an enclosed/limited reservoir avoids problems due to gas bubbles in the liquid circuit. A common ground connection was also formed by connecting the ground of the external power supply for CEC separation (HV_2), the shielding of the ESI power supply (HV_1) lead cable, and the mass spectrometer.

Preparation of open-tubular capillary column coated with Lupamin

Open-tubular capillary column was coated with Lupamin as stationary phase. Capillary was pretreated by washing for several minutes with water and acetone, respectively, and then dried at 105 °C for 2 h. The pretreated capillaries were coated with a 0.5% Lupamin aqueous solution infused for 3 h by a syringe pump, and then washed for 1 h with DI water to remove the uncoated Lupamin. The coated capillary was ready to use for fabrication of the CEC-ESI device.

Measurement of electroosmotic flow rate

The rate of EOF was measured by weighing the electrolyte mass transferred from the CEC-ESI capillary. The spray capillary tip was inserted into the middle of a measurement capillary (6 cm, 700 μm i.d., 850 μm o.d.), which had been weighed empty. After applying the electrospray and separation voltages to the CEC-MS device for 5–10 min, the measurement capillary and the resultant solution collected inside it were weighed. The flow rate of EOF (Q) was calculated by converting the mass of the collected liquid to volume (v, $v = w/d$, w: weight, d: density of water) and dividing this value by the total collection time (t), $Q = v/t$.

CEC-ESI-MS

A triple-quadrupole mass spectrometer (Micromass QuattroLC, Waters) equipped with a nanoelectrospray ionization source (NewObjective) was used for all CEC-MS experiments. The sheathless interface with Nafion tubing junction assembly was mounted on the nanoelectrospray source as shown in Fig. 2. The spray capillary tip was set approximately 2–5 mm away from the MS orifice with around 30–45° angle, and fine positioning of spray tip was achieved by using the PicoView *XYZ* stage (PV300, NewObjective). The reservoir was filled with the running electrolyte, and the spray voltage from the MS power supply was applied via a platinum electrode immersed in the electrolyte. The applied spray voltage varied between 2.2 and 2.8 kV as needed for optimum spray stability. The ESI source parameters were set as follows: source temperature, 125 °C; cone voltage, 15 V; extractor voltage, 4 V; and r.f. lens, 0.3 V. The spray was monitored by a CCD camera (Model XC-ST30, Sony), and the video output was displayed with a Sony monitor. Images of spray displayed on the monitor were taken by a digital camera (Nikon Coolpix 880). Data acquisition and processing of the electrochromatograms and mass spectra were carried out by the MassLynx version 4.0 software (Waters). On-line ESI-MS was performed in the positive ion mode, typically using an ESI voltage of 2.5 kV. The CEC separation voltage was applied by an external power supply (Matsusada Precision Devices Inc., Japan) in the range of −20 to 20 kV depending on the EOF direction required. The net electrical field across the separation capillary (separation voltage) is the applied voltage from the external power supply (HV_2) minus the ESI voltage (HV_1): $HV_2 - HV_1$. The sample was hydrodynamically injected by inserting the capillary into the sample vial and elevating the vial by 10–15 cm high for 20 s.

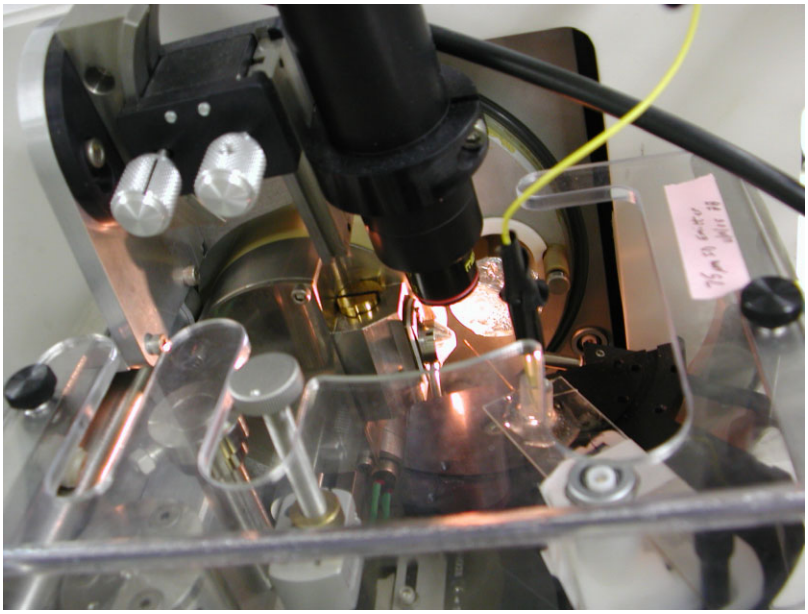

Figure 2. Sheathless CE/CEC-ESI-MS interface mounted on PicoView nanoelectrospray assembly for Micromass QuattroLC MS.

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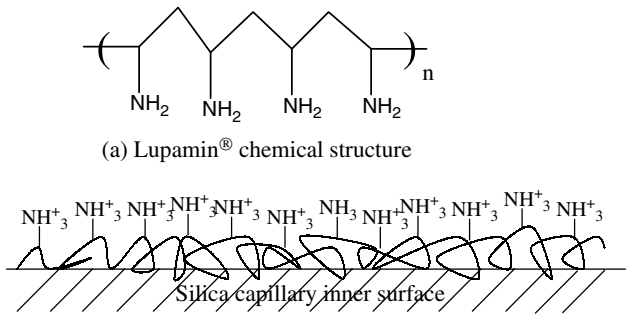
RESULTS AND DISCUSSION

Coating of Lupamin on the capillary inner wall

Polyamine (PolyE-323)-coated capillary has been reported as a simple, fast, and efficient way to prepare an open-tubular capillary column for CE-MS.[19] Lupamin is a high-molecular-weight linear PVAm with the chemical structure shown in Fig. 3(a). The polymer can be coated on the silica capillary inner wall by adsorption from an aqueous solution, as shown in Fig. 3(b).[20] The adsorption of PVAm on silica in water is determined by electrostatic forces rather than by specific chemical interactions.[21] The PVAm forms an entangled positively charged layer with $-NH_3^+$ groups on the surface of the capillary inner wall. Recently, we used Lupamin to modify the silica particles as virus capture media with positive surface charge.[22] When Lupamin was adsorbed on to the silica particle surface, the zeta potential of silica particles changed sign from negative to positive. The zeta potential of Lupamin-coated silica particle was pH dependent and was measured as 40 mV at pH 3 with a zeta potential analyzer. This suggested that it was an excellent material for changing the EOF from a silica surface and for offering chromatographic retention and selectivity as a stationary phase. For the analysis of peptides and proteins, aminopropyl silylated (APS) capillary has been used to minimize zone broadening due to adsorption effects.[15,23] However, the derivatization of the capillary wall with APS is tedious, and the on-capillary silylated chemical reaction often results in low silylation efficiency due to the difficulty of controlling nonaqueous conditions. This paper presents the first application of Lupamin in capillary column technology. This stationary phase offers many advantages such as easy preparation, low cost, high coating efficiency, and durability for CE/CEC separation. Additionally, as discussed in the following sections, linear PVAm not only provides strong reversed EOF for ESI, but it also improves chromatographic selectivity for the separation of amino acids and peptides. The device proved to be very durable, withstanding several weeks (~hundreds of CEC-ESI runs) of use without any observable deterioration in either CE separation or electrospray performance.

Sheathless nanospray device, spray stability, and electroosmotic flow rate

A simple, sheathless device, as illustrated in Fig. 1, was developed for the direct separation and detection of amino acids and peptides by on-line CEC-ESI-MS. This device offers many advantages over sheath-flow and liquid junction interfaces, including ease of fabrication, ruggedness, durability, and no dilution effects. As Nafion tubing is a proton-transferring membrane, there is only ionic transfer for electric field penetration. Because the electrolyte in the external reservoir was the same as that in the separation capillary, there was no net electrolyte addition, no analyte dilution, and no production of ionic boundaries. In addition, because the CEC separation was performed using open-tubular capillary column, there was minimal opportunity for cross contamination due to carry-over effects from one separation to another. Moreover, an open-tubular capillary column can offer high flow rate of EOF, which improves the formation of a stable electrospray. As shown in Fig. 4(a), a very stable Taylor cone was generated at the spray tip of the Lupamin-coated CEC-ESI device, when 1 M acetic acid was used as the running electrolyte, and 2.5 and −17.5 kV were applied as the spray and separation voltages, respectively. Although the droplets in the spray plume cannot be seen, the generated ion current was sufficient for MS detection. Using the method described in 'Measurement of Electroosmotic Flow Rate', the flow rate of EOF under these experimental conditions was determined to be 140 nl/min. The direction of EOF went from cathode to anode. In order to generate a stable electrospray from this device, it was necessary to produce an EOF rate of greater than 50 nl/min in the separation capillary column. With the tubing diameters used herein, the recommended working flow rate of EOF for CE/CEC-ESI-MS was in the range from 50 to 200 nl/min. When a negative polarity spray voltage was applied, as shown in Fig. 4(b), a seemingly stable electrospray with a well-formed, fog-like plume was observed. However, the only ions detected in the mass spectrum appeared to arise from solvent clusters.



(a) Lupamin® chemical structure

(b) Lupamin® polymer layer adsorbed onto silica capillary surface

Figure 3. (a) Lupamin chemical structure, (b) Lupamin polymer layer adsorbed onto silica capillary surface.

Separation and detection of amino acids and peptides on the coated capillary

A representative sample containing two amino acids (Phe and Leu) and three peptides (Gly-Leu, Gly-Phe, and Leu-Gly-Gly) was employed as test mixture for CEC separation and ESI-MS detection. Figure 5 shows (a) extracted ion electrochromatograms of $[M + H]^+$ ions at m/z values 166 (Phe), 132 (Leu), 246 (Leu-Gly-Gly), 223 (Gly-Phe), and 189 (Gly-Leu), (b) overlaid ion electrochromatogram of these 5 components, and (c) mass spectra obtained from CEC-ESI-MS. The separation conditions were as follows: 1 M acetic acid as running electrolyte, Lupamin-coated capillary as separation column, spray voltage = +2.5 kV and separation voltage = −17.5 kV. As shown in Fig. 5(b), an excellent baseline separation was achieved for all five components. Analytes possess a net positive charge under this acidic condition and migrate toward the cathode (the electrode applied negative electric field); however, the stronger EOF than electrophoretic mobility of analytes drives analytes through the separation column toward the electrospray tip. As described in 'Coating of Lupamin on the Capillary Inner Wall', Lupamin-coated capillary at acidic condition possesses a high density of positively charged amino groups on the inner wall of the capillary. Positively charged amine groups in PVAm play three important roles: (1) they

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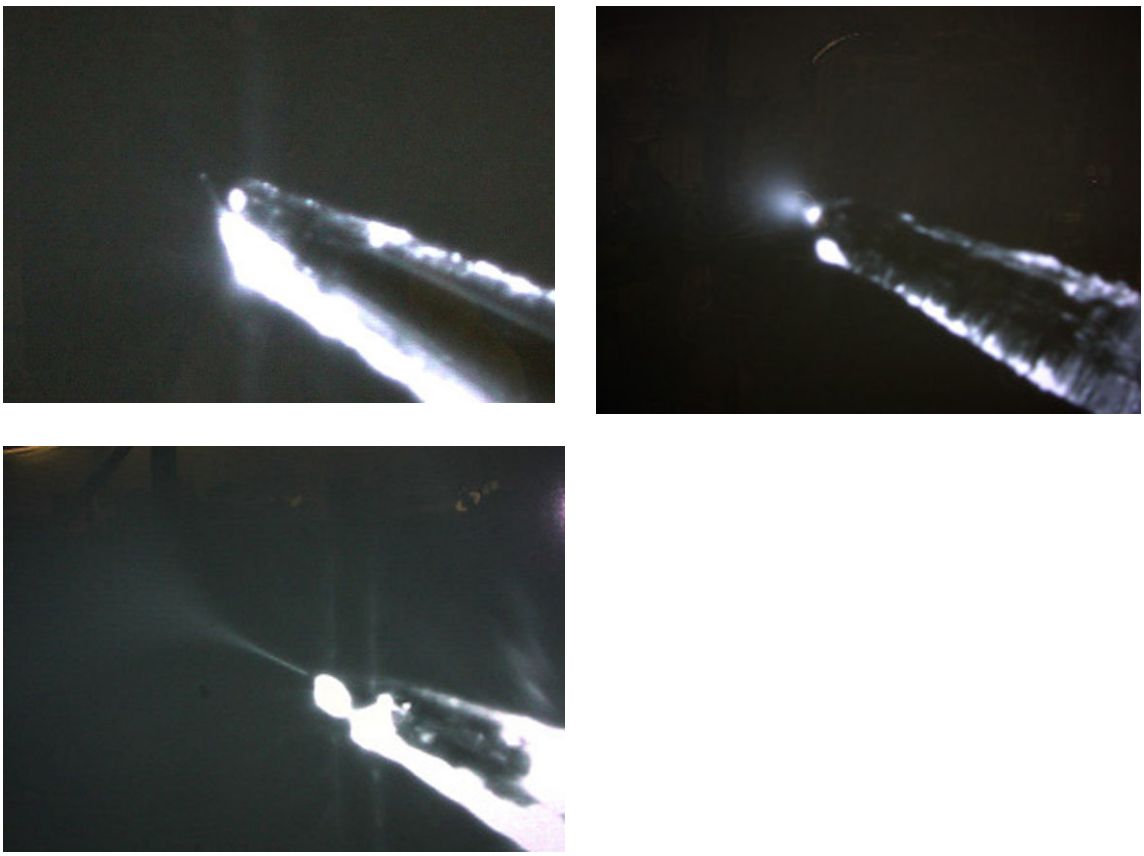

Figure 4. Imagings of electrospray and Taylor cone. (a) Lupamin-coated open-tubular capillary, running electrolyte: 1 M acetic acid, spray emitter: sharply tapered capillary, electrospray voltage: 2.5 kV and separation voltage: −17.5 kV; (b) Lupamin-coated open-tubular capillary, running electrolyte: 0.6 mM ammonium acetate, spray emitter: sharply tapered capillary, electrospray voltage: −2.7 kV and separation voltage: 2.7 kV; (c) uncoated bare silica capillary, spray emitter: sharply tapered capillary, electrolyte: 20 mM NH_4OH, 2.65 kV spray voltage and +12.35 kV separation voltage.

provide a strong reversed EOF for driving mobile phase and analytes from inlet end of separation capillary (cathode) to the electrospray tip, (2) they work as stationary phase by allowing chromatographic interactions between analytes and PVAm, and (3) they eliminate the adsorption of analyte to the capillary wall. A sufficient flow rate of EOF is necessary for driving the electrolyte through separation capillary for CEC separation and for generating a stable electrospray for the detection of ESI-MS in CE/CEC. Lupamin-coated capillary could generate nano-scaled and reversed EOF for ESI up to a flow rate of over 200 nl/min, which is sufficient for CEC separation and ESI-MS detection. At the experimental conditions in Fig. 5, the flow rate of EOF was determined to be 150 nl/min. Owing to the electrostatic attraction between the positively charged analytes and negatively charged silanol groups of the silica capillary, adsorption of analyte on the inner wall of bare silica capillary is a major problem for CE/CEC analysis of peptides and proteins in acidic conditions.[15,23] To solve this adsorption problem, APS-modified[15,23] and polyacrylamide-coated capillaries[13] have been used. In this work, the analyte adsorption problem was eliminated with the Lupamin-coated capillary, thus demonstrating that this simple and efficient approach is effective for reversing EOF and facilitating the separation of analytes.

Separation behavior of amino acids and peptides in uncoated silica capillary

To demonstrate the function of PVAm as a chromatographic stationary phase and to obtain some evidence of the electrochromatographic mechanism, an uncoated bare capillary was used for the analysis of the same sample by CEC-ESI-MS. At first, we investigated the separation behavior on bare silica capillary under similar conditions with lupamin-coated capillary using 1 M acetic acid as running electrolyte, and applying +2.65 kV spray voltage. When −17.5 kV was applied at the separation capillary, the reversed EOF contributed from the protonization of silanol groups of bare silica capillary was so weak that a stable electrospray could not be generated. When +16.35 kV of voltage was applied to separation capillary, the resulting electrospray was extremely weak and unstable, and only the highest concentration analyte ions $[Leu + H]^+$ and $[Leu\text{-}Gly\text{-}Gly + H]^+$ at m/z 132 and m/z 246, respectively were observed in the mass spectrum at retention times of ~18 min (Fig. 6(a)). A comparison of Figs 5(a) and 6(a) shows that the signal counts of m/z 132 peaks in coated and uncoated capillary column are on the orders of 10^8 and 10^6, respectively. This suggested that analyte adsorption occurred on the uncoated surface of the capillary and that sample diffusion may have resulted from the weak EOF rate under this acidic condition. Therefore, the uncoated capillary is not suitable for the analysis of these

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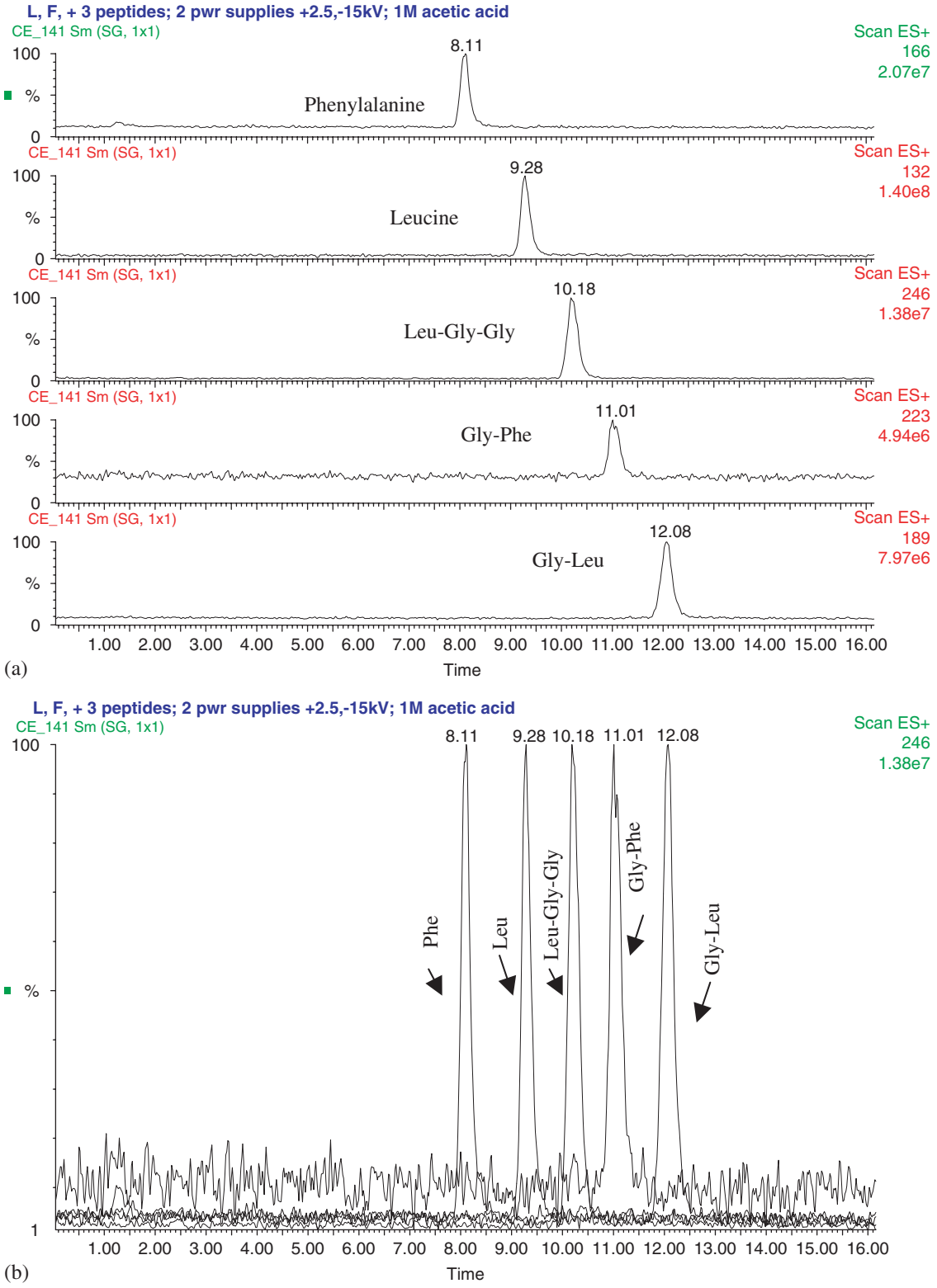

Figure 5. (a) Extracted ion electrochromatograms of *m/z* $[M + H]^+$ 166 (Phe), 132 (Leu), 246 (Leu-Gly-Gly), 223 (Gly-Phe), and 189 (Gly-Leu), (b) overlaid ion electrochromatogram and (c) mass spectra obtained from CEC-ESI-MS. Running electrolyte: 1 M acetic acid, Lupamin-coated capillary column, 2.5 kV spray voltage, and −17.5 kV separation voltages.

amino acids and peptides, and the low detection sensitivity is likely due to sample adsorption and low mass transfer to the emitter tip.

Further, we examined the separation behavior on uncoated capillary using 20 mM ammonium hydroxide as the running electrolyte. When +2.65 kV spray and +12.35 kV separation voltages were applied, as shown in Fig. 4(c), a very strong spray with long liquid jet and cone-like plume appeared indicating a strong EOF with a flow rate measured to be ~180 nl/min. Figure 6(b) shows the extracted electrochromatograms of $[M + H]^+$ ions at *m/z* values 166 (Phe), 132 (Leu), 246 (Leu-Gly-Gly), 223 (Gly-Phe), and 189

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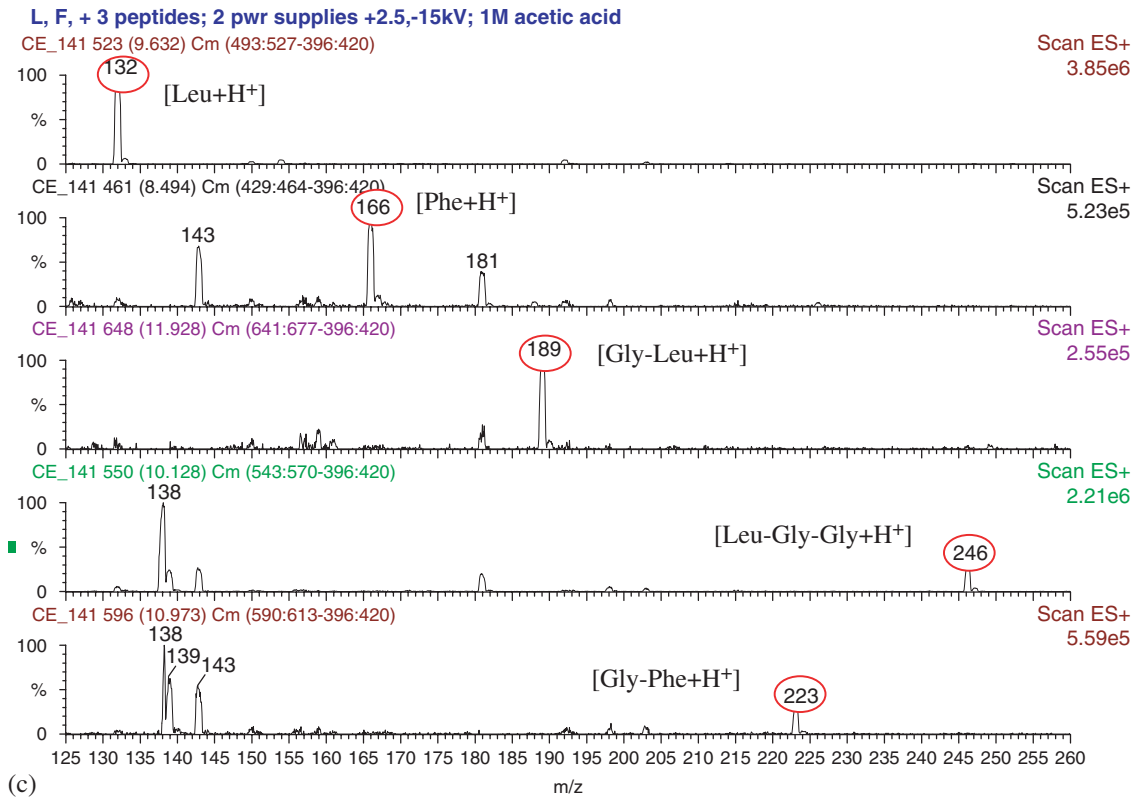

Figure 5. (*Continued*).

(Gly-Leu) obtained from CE-ESI-MS analysis. Notice that all analytes showed the same retention time. Decreasing the separation voltage shifted only the migration times of analytes without improving the separation. Thus, by comparing Figs 5 and 6, it was concluded that amino groups in PVAm play an important role in providing selectivity as a chromatographic stationary phase for resolving amino acids and peptides.

Effect of running electrolytes at different concentrations and pH on the separation behavior

Electrolyte composition and pH are important factors for controlling separation selectivity, because these parameters could be used to change both the rate of EOF and the chemical nature of stationary phase and residual silanol groups on the inner wall of the capillary. The effect of acetic acid concentration on separation behavior was investigated. When acetic acid concentration was decreased to 10 mM and 2.5 kV spray voltage and −17.5 kV separation voltage were applied, retention times of Phe, Leu, Gly-Phe, Leu-Gly-Gly, and Gly-Leu were 4.69, 4.86, 5.89, 6.18, and 6.36 min, respectively. These conditions provide little separation between Leu and Phe, poor resolution among the three peptides, and baseline separation between the amino acids and peptides. When 1 mM ammonium acetate was used as running electrolyte and 2.5 kV spray voltage and −17.5 kV separation voltage were applied, retention times of Phe, Leu, Gly-Phe, Leu-Gly-Gly, and Gly-Leu were 4.38, 4.40, 4.58, 4.62, and 4.60 min, respectively. When 10 mM ammonium acetate was used as the running electrolyte, 2.5 kV spray voltage and +12.5 kV separation voltage were applied. As shown in Fig. 7(a), the five components cannot be resolved owing to the effect of uncoated residual silanol groups on the inner surface of the capillary. To demonstrate further the effect of residual silanol groups, 20 mM ammonium hydroxide was used as the running electrolyte. Because the electrolyte is at a high pH condition, amino groups in PVAm will dissociate the proton from positively charged $-NH_3^+$ and become neutral $-NH_2$ groups. Meanwhile, residual silanol group will lose a proton from –Si –OH to form —Si $-O^-$ groups, which contribute to the formation of EOF. Therefore, a positive polarity separation voltage should be applied to generate an electrospray and to drive analytes to the MS detector. As shown in Fig. 7(b), the separation cannot be achieved even by using Lupamin-coated capillary at extreme alkaline conditions, as active sites for chromatographic retention in this case are silanol groups and not PVAm. These results suggest that $-NH_3^+$ groups in PVAm play an important role as a stationary phase for interactions with the analytes.

CONCLUSIONS

A novel chip-scale, sheathless CEC-ESI device with nanoflow and Nafion tubing junction has been developed and successfully tested for the separation and detection of some amino acids and peptides by CEC-ESI-MS. This device offers many advantages, such as ease of fabrication, ruggedness, durability, and no dilution effects, over existing devices, which require sheath flow or a liquid junction interface. This report offers the first use of Lupamin-coated open-tubular capillary column for CEC separation. This column has several merits including easy preparation, low cost, and good reliability. Positively charged amino groups in PVAm

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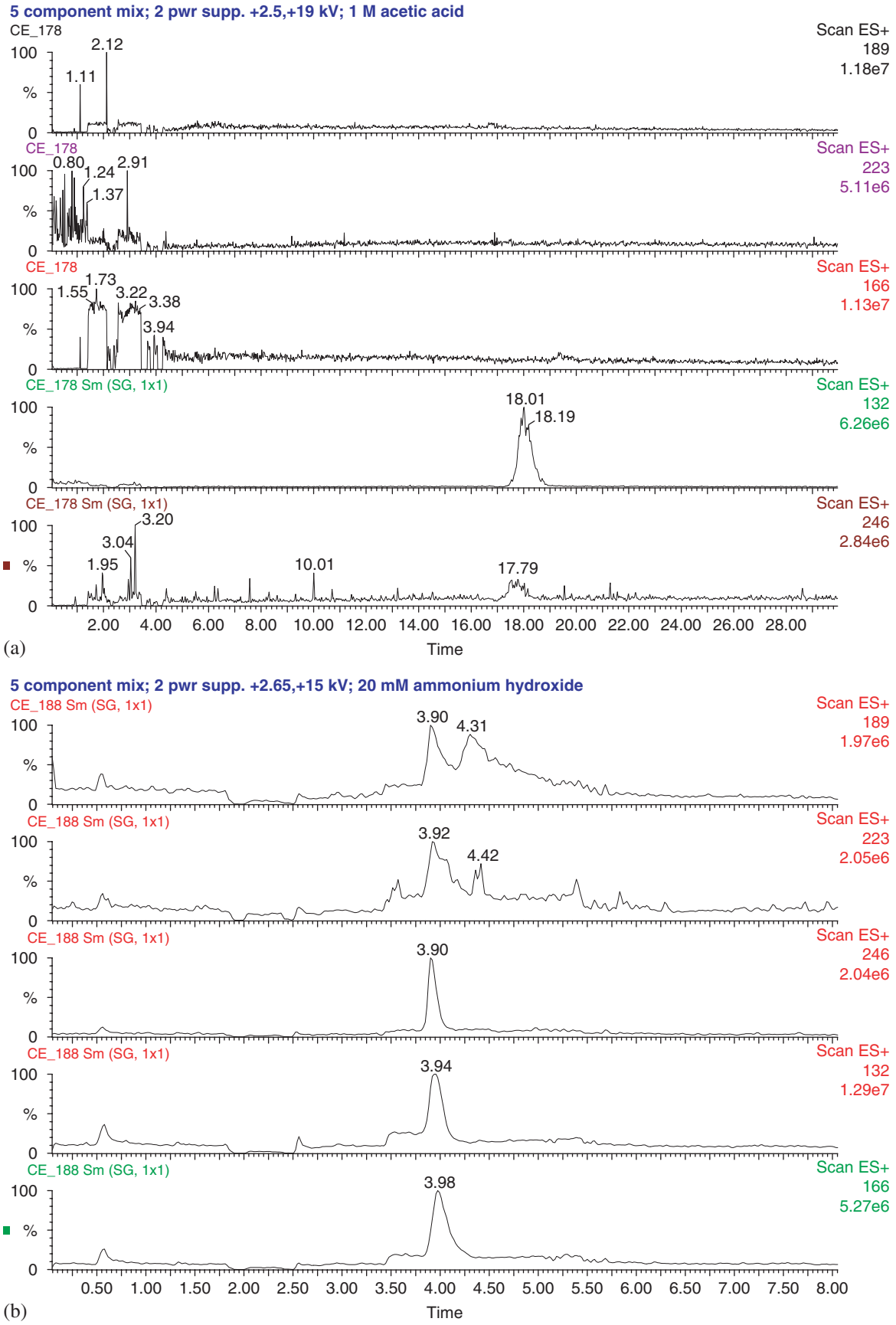

Figure 6. Extracted ion electrochromatograms of *m/z* $[M + H]^+$ 166 (Phe), 132 (Leu), 246 (Leu-Gly-Gly), 223 (Gly-Phe), and 189 (Gly-Leu) obtained from CE-ESI-MS with bare silica capillary. (a) Running electrolyte: 1 M acetic acid, 2.8 kV spray voltage, and 12.2 kV separation voltage; (b) Running electrolyte: 20 mM ammonium hydroxide, 2.65 kV spray voltage, and 12.35 kV separation voltage.

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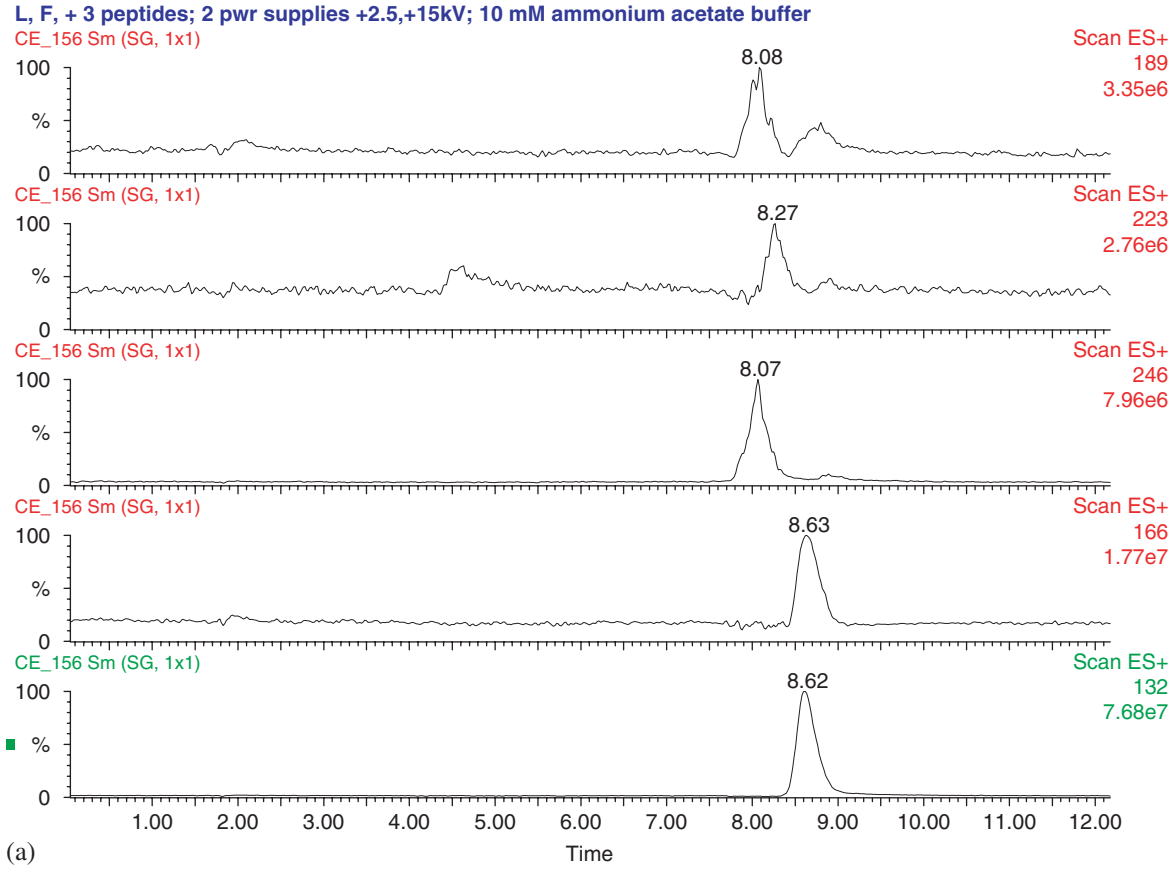

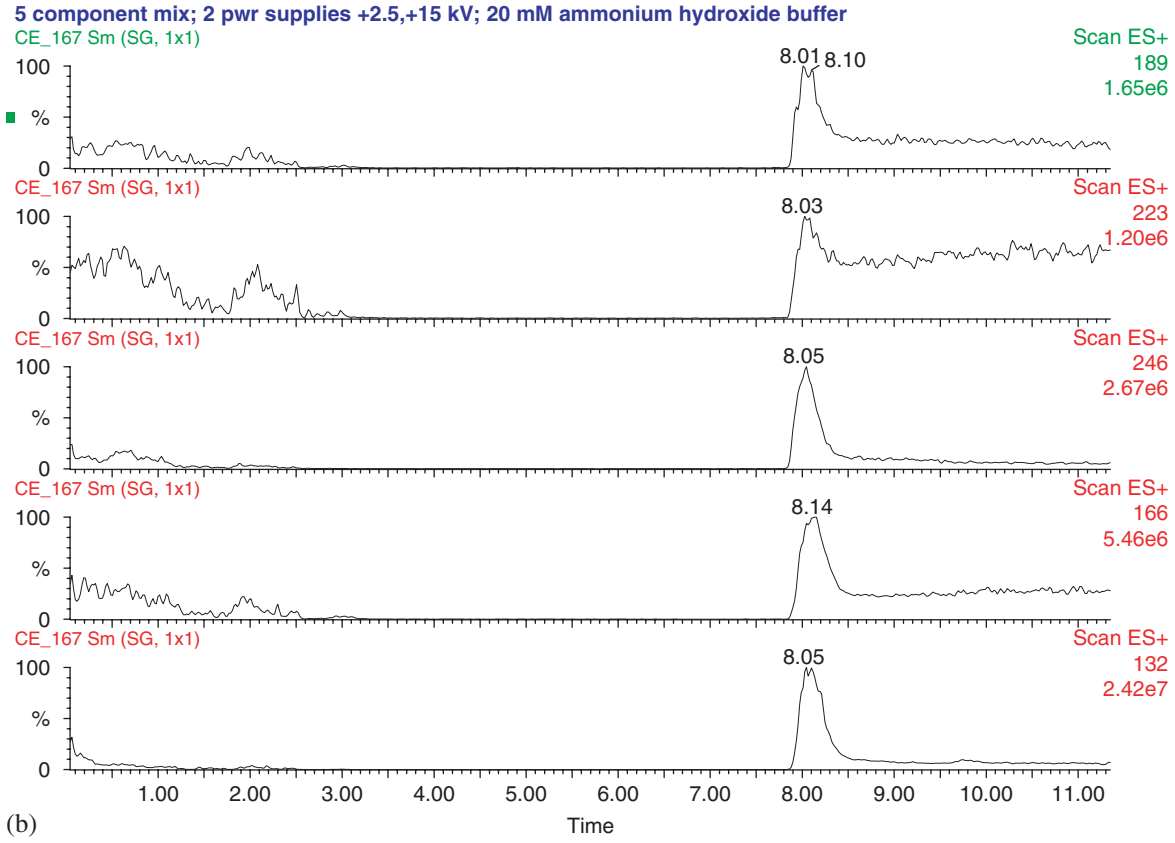

Figure 7. Extracted ion electrochromatograms of *m/z* $[M + H]^+$ 166 (Phe), 132 (Leu), 246 (Leu-Gly-Gly), 223 (Gly-Phe), and 189 (Gly-Leu) obtained from CE-ESI-MS with Lupamin-coated silica capillary. (a) Running electrolyte: 10 mM ammonium acetate, 2.5 kV spray voltage, and 12.5 kV separation voltage; (b) Running electrolyte: 20 mM ammonium hydroxide, 2.65 kV spray voltage, and 12.35 kV separation voltage.

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in acidic conditions play three important roles: (1) they provide a strong reversed EOF for driving the mobile phase and analytes from the inlet end of the separation capillary to the electrospray tip; (2) they function as a stationary phase by improving specific chromatographic selectivity for resolving amino acids and peptides; and (3) they eliminate the adsorption of analyte resulting from the electrostatic adsorption in the case of bare silica capillary. This integrated, sheathless CEC-ESI device and Lupamin-coated open-tubular capillary technology are expected to be applied in routine separation and identification of amino acids and peptides by CEC-ESI-MS in academic and industrial laboratories.

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