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Evaluation of an electrospray interface for capillary electrophoresis-time-of-flight mass spectrometry[1]

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Abstract

The electrospray technique has developed into a widely used ionization source for interfacing capillary electrophoresis (CE) to mass spectrometry (MS). However, its implementation is not always straightforward. A large number of factors have been found to be important contributors to the production of a high quality spray, and the efficient transfer of analytes from the CE column into the mass spectrometer can become, in certain cases, troublesome. An electrospray device which can accommodate operation with a liquid sheath, nebulizing gas and make-up gas, as well as operation without a liquid sheath in the microelectrospray mode, was constructed. The electrospray source was evaluated for efficient CE separations, which require the best performance. © 1997 Elsevier Science B.V.

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1. Introduction

Capillary electrophoresis (CE) has experienced exponential development during the 1980s, initiated by Jorgenson and Lukacs [1,2], and coworkers [3,4] who also set the standard for the highest performance of the technique by generating more than 1 000 000 plates in less than 20 min, and more than 100 000 plates in less than 1 min. Later developments expanded the applicability of CE and its related techniques to not only ionic species, but also nonionic (both polar and nonpolar) as well. Low- and high-molecular-mass compounds are amenable to analysis. On-line interfacing of such a high efficiency separation method to specific detection techniques, such as mass spectrometry (MS), has consequently become a tempting and challenging task.

The first papers involving the coupling of CE to MS were published in 1987–88 [5–8]. Since then, numerous papers have been published concerning on-line CE–MS experiments, using mainly quadrupole, ion trap, or ion cyclotron resonance MS instruments and electrospray or continuous flow fast atom bombardment (CF-FAB) interfaces. Low sensitivity, increased background noise, and less applicability to high-molecular-mass compounds experienced when using CF-FAB [9], permitted electrospray to dominate the field of CE–MS interfacing.

Electrospray, first proposed by Dole et al. [10,11],

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mechanistically studied by Iribarne and Thomson [12], and then promoted by Fenn and coworkers [13–15], is a very simple and efficient ionization technique which takes ions directly from the liquid into the gas phase. It is applicable to almost the same class of analytes as is CE: ionic or polar, and low- or high-molecular-mass compounds. Only neutrals are not amenable to analysis. The widespread use of electrospray is due to its multiple advantages: high ionization efficiency, multiple charging of molecules which yields ions of mass-to-charge ratios compatible with the operating mass range of most analyzers, soft nature of the ionization technique with no, or little, fragmentation, and ultimately, the fact that it is an atmospheric pressure ionization (API) source, which avoids vacuum-induced laminar flow in the CE capillary which can destroy the separation efficiency.

The interfacing of capillary electrophoresis to time-of-flight mass spectrometry (TOF-MS) was reported in the last three years [16–22], and these studies were conducted using an electrospray ionization (ESI) source. The desirable attributes of TOF-MS [23], such as speed, unlimited mass range, high ion transmission efficiency, high duty cycle and sensitivity, most closely match the requirements of CE, and qualify it as the detection tool of choice for CE.

This paper describes the design of an electrospray ion source for CE–TOF-MS, and presents an evaluation of its performance and limitations with respect to two of the most important aspects of interfacing: preservation of separation efficiency and resolution.

2. Experimental

2.1. Reagents

Standard solutions were prepared in HPLC grade solvents. Methanol and water were purchased from Mallinckrodt (Chesterfield, MO, USA). Glacial acetic acid was obtained from EM Science (Gibbstown, NJ, USA). All peptides were purchased from Sigma (St. Louis, MO, USA). Samples were prepared in methanol–water (50:50, v/v) or methanol–water–acetic acid (50:50:0.03, v/v).

2.2. Instrumentation

2.2.1. Capillary electrophoresis

The research was carried out using a Crystal CE 300 system (ATI, Madison, WI, USA). The UV detector was an Applied Biosystems (Foster City, CA, USA) Model 785A detector. Coated and uncoated fused-silica capillaries, 190 μm O.D.×50 μm I.D. of various lengths were used to perform the separations. CElect-Amine capillaries were supplied by Supelco (Bellefonte, PA, USA). Continuous infusion of the analyte solutions was accomplished using a Harvard 22 syringe pump (South Natick, MA, USA).

2.2.2. Time-of-flight mass spectrometry

The time-of-flight mass spectrometer was designed and constructed at Brigham Young University. A detailed description of the TOF instrument is given in a previous paper [24].

2.2.3. Electrospray interface

The liquid sheath electrospray (Fig. 1) was constructed following the basic features of the first liquid sheath ESI source [8] designed in 1988. In addition to the liquid sheath and make-up gas capabilities, our design incorporates the possibility of a nebulizing gas as well [6].

The device consists of two main Tefzel chambers, 1 and 2, held together by nylon screws. The electrical contact for electrospray operation, which also defines the electrical contact for the CE buffer at the detection end, or interface end, is realized through the liquid sheath supplied through the electrospray needle, 3. Electrospray needles were purchased from Scientific Instrument Services (Ringoes, NJ, USA), or were prepared from stainless-steel needle tubing (Hamilton, Reno, NV, USA) which was mechanically tapered at the spray tip. The gauge of the ESI needle was chosen such that its I.D. matched as close as possible the O.D. of the fused-silica capillary used for the CE separation. Gauge 26 and 27 needles were used. The electrospray voltage is applied through a spring-loaded contact to the stainless steel tubing which supplies the nebulizing gas, 4. Good electrical connection to the electrospray needle is provided through a removable contact wire between tubing 3 and 4 in the electrospray chamber 1. The constant

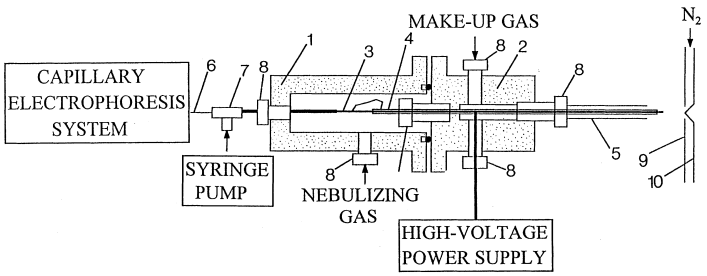

Fig. 1. Schematic diagram of the electrospray source. (1) Electrospray chamber 1 (Tefzel); (2) electrospray chamber 2 (Tefzel); (3) electrospray needle; (4) nebulizing tubing (stainless-steel); (5) make-up gas tubing (glass); (6) CE capillary; (7) PTFE T-union; (8) nuts, tubing connectors (Delrin); (9) interface plate; (10) nozzle.

liquid flow for the sheath electrode is supplied by a syringe pump through a small PTFE T-union, 7. The fused-silica capillary from the CE system, or from the syringe pump, was introduced through the same T-union, 7, until it protruded slightly from the ESI needle.

The nitrogen nebulizing gas is supplied through the electrospray chamber 1 at flow-rates dictated by the optimum operating conditions. The gauge of the stainless-steel tubing which supplies the nebulizing gas was also chosen such that its I.D. matched closely the O.D. of the electrospray needle. This design allows for an additional make-up gas supply, when necessary, through electrospray chamber 2 and a 1/8 in. O.D. glass tube. Chambers 1 and 2 are insulated from each other, so that mixing of the nebulizing and make-up gases cannot occur. The purpose of the make-up gas, sulfur hexafluoride, is to suppress any electrical discharge. The electrospray device is about 4.50 in. long and 1.25 in. in diameter (1 in.=2.54 cm). Except for the ESI needle and the nebulizing gas tubing, it was constructed entirely from electrically insulating materials.

An electrospray focusing lens, the interface plate 9, is placed in front of the mass spectrometer sampling nozzle, 10. A countercurrent flow of heated nitrogen, the curtain-gas, is supplied between the nozzle and the electrospray focusing lens. The curtain-gas, as is well known, helps with the desolvation of the electrosprayed droplets, minimizes ion-solvent cluster formation, and prevents the nozzle orifice from contamination or clogging by microparticulates. A multiple axis micrometer (Newport, Irvine, CA, USA) allows for precise positioning of the ESI needle relative to the sampling nozzle inside the ion source.

Optimization of the electrospray technique in order to spray directly from the CE capillary at very low flow-rates (0–300 nl min^{-1}), without the application of a liquid sheath (i.e., the microelectrospray mode), is very advantageous since the microelectrospray is a much more efficient ion source [25,26]. Smaller droplets are initially produced, and much more efficient desolvation of ions and ion evaporation are achieved, which finally results in better sensitivities and low background noise. The high electric field strength produced at the sharp capillary tip allows highly aqueous solutions to be electrosprayed at relatively low voltages without arcing. Since plugging of the capillary tips (3–5 μm) used typically in the micro/nanoelectrospray may occur frequently, we have chosen to perform some studies with 50 μm I.D. capillaries drawn out to 10–20 μm I.D., and 40–60 μm O.D. (Fig. 2). The ESI tips were prepared by pulling out fused-silica tubing between two electrodes. A model M100 manipulator device (Polymicro Technologies, Phoenix, AZ, USA) allowed for precise control of the current and the time the current is applied to the electrodes during preparation of the tips.

Even though somewhat stable gold coating technologies have been described in the literature [27,28], the procedure is still time consuming, and manipulation of the capillaries requires great care, skill and patience. We attempted to avoid the gold coating approach, and applied the electrospray voltage through a metal union placed close to the CE separation capillary tip (1–1.5 cm) in order to

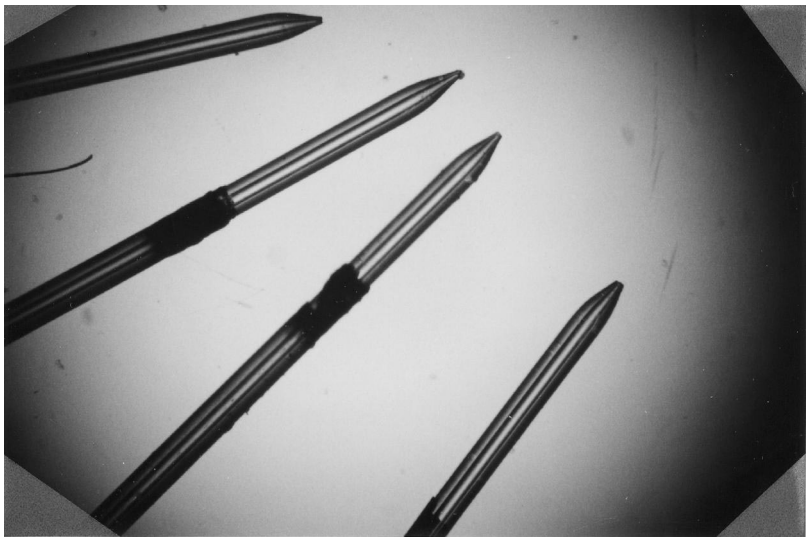

Fig. 2. Photograph of the microspray needles.

provide minimal loss of efficiency (Fig. 3). The metal union consisted of a 1 cm long stainless-steel needle, of the same kind as those used for the ESI needle in the liquid sheath source.

The sharp ESI tip was cut to 1–1.5 cm and attached to the separation capillary (of the same I.D. and O.D.) by pushing the ESI tip and the capillary against each other inside the metal tubing/union, and cementing the fused-silica to the metal tubing with epoxy glue. The ESI voltage was applied to the metal union. Since a perfect cut and assembly of the two fused-silica pieces is very hard to achieve, and since less than perfect connection may have a negative effect on the separation efficiency and resolution, we implemented a new procedure to establish electrical contact with the eluent. By scratching the capillary at approximately 1.5 cm from the drawn-out tip, without detaching completely the tip, but letting it stay attached through the polyimide coating, and physically supporting it with the glued metal union, there was a sufficient leak of the eluent to provide electrical contact with the inside wall of the metal tubing.

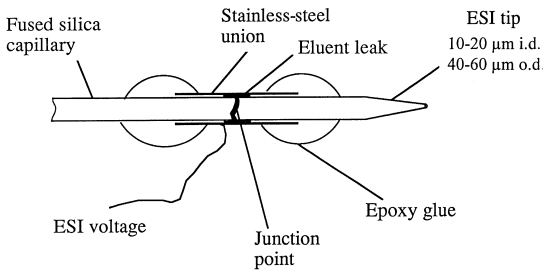

Fig. 3. Schematic diagram of the microspray set-up.

Since bubbles were sometimes produced at the metal/liquid interface (originating either from the initially trapped air inside the union, or due to electrochemical processes), we used another configuration as well, in which the metal tubing was replaced by a 360 μm O.D.×200 μm I.D. fused-silica capillary which allowed visualization of the junction point. With this union it was possible to observe that when there was liquid flow through the fused-silica capillary supplied by the syringe pump, the eluent leak at the cut or scratch point on the fused-silica tubing produced a small (2–3 mm) liquid plug that ensured an electrical contact with the metal wall. Air seemed to remain always trapped inside the union. However, during CE operation, the EOF seemed not to be sufficient to ensure a stable contact to the metal union.

A microscope (Edmund Scientific, Barrington, NJ, USA) positioned in front and at an oblique angle above the source permitted close evaluation of the spray characteristics. Magnification settings of 12 or 25 on the microscope proved to be sufficient. A fiber

optic illuminator (Edmund Scientific) was used to shine light on the spray. A plastic window on the ion source allowed direct observation of the ESI needle position and the spray.

3. Results and discussion

3.1. Spray stability

One of the major problems in the beginning of this work was to obtain a stable spray with both liquid sheath and microelectrospray configurations. Some of the most important factors which contributed to this situation were the following:

(1) The electrospray voltage. The electrospray voltage had a critical influence on both spray stability and signal intensity. We observed that lowering the ESI voltage after the onset of the electrospray and after stabilization or equilibration of the liquid flows was, in most cases, beneficial. When the microscope was added to the system, we observed that the cause of low or non-existent signal was the simple fact that the spray was not oriented straight ahead of the ESI tip, but towards the side of the source, off-axis at 30–45°. Sometimes a multiple spray was observed as well. Lowering the ESI voltage brought back a straight alignment between the spray, the Taylor cone and the spray needle, and increased the signal intensity.

(2) The ESI tip quality. The shape uniformity and the surface quality and finish of the ESI tip were also partially responsible for the above problems.

(3) The ESI tip position in the ion source. The liquid sheath source was usually positioned at about 10–12 mm from the sampling nozzle, and slightly off-axis (2–3 mm). The microspray was positioned closer at about 4–5 mm from the sampling nozzle, especially when seeking stronger signals. However, it was much more difficult to obtain a stable spray under these conditions. If the signal intensity was not the major objective, the microspray was positioned at 7–8 mm away from the nozzle orifice. Sometimes it helped to position it 1–2 mm off-axis as well. Lowering the ESI tip-nozzle distance from 7–8 mm to 4–5 mm resulted in 60–100% increase in signal intensity. It was always critical to apply the right ESI voltage for a given nozzle orifice–spray tip distance.

(4) The position of the fused-silica capillary in the ESI needle. It is well documented that the fused-silica capillary must protrude only a little (less than 0.2 mm) from the ESI needle [8]. We experienced the same result. Improper positioning resulted in either complete signal loss or spray instability. Observation under the microscope again helped us to elucidate this condition.

3.2. Signal reproducibility

All of the above-mentioned factors ultimately affected the ability to produce a reproducible signal from one day to another. On the average, variations within 20–30% of an absolute signal magnitude were common. Only with patient optimization was it possible to bring these fluctuations to less than 15–20%. Initially we assumed that lack of precise control in the fabrication of the microspray tip or ESI stainless steel needle was the main factor. However, it was interesting to observe that sometimes during the same day with the same tip, and under exactly the same conditions, we experienced the same variations in signal intensity.

3.3. Liquid flows

For continuous infusion experiments, we operated the electrospray under conditions similar to a CE run, i.e., at less than 300 nl min^{-1} with both the microspray and liquid sheath source. When the CE separations were conducted in conjunction with the microspray source, a slight pressure, 10 mbar (1 kPa), was applied to the inlet buffer vial in order to help stabilize the spray under low electroosmotic flow conditions. Preliminary experiments were conducted with the UV detector to determine if the applied inlet pressure would compromise the separation efficiency, because of induced laminar flow in the CE capillary. In the case of the separation of enkephalins with nonaqueous CE [24], the applied pressure actually improved the efficiency (Fig. 4). For the leucine enkephalin peak, the efficiency increased from approximately 160 000 plates to approximately 200 000 plates, and the peak intensity increased two times, when 1 kPa pressure was applied to the inlet vial. However, in the case of other analytes, an increase in peak intensity was not

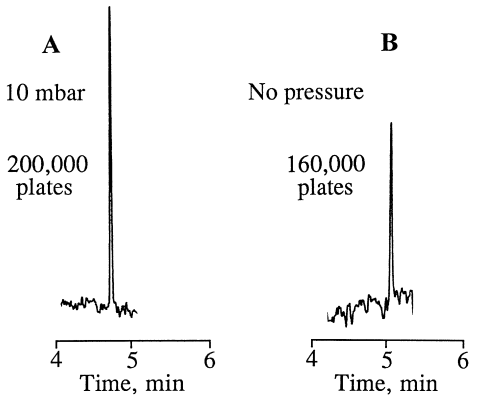

Fig. 4. Effect of CE inlet vial pressure on efficiency. Sample: solution of 40 μ*M* leucine enkephalin in methanol–water–acetic acid (50:50:0.03, v/v). Conditions: 85 cm×50 μm I.D. uncoated fused-silica capillary, acetonitrile–water–acetic acid (50:50:0.3, v/v), 30 kV, 0.3 μA, 2 kPa×0.05 min injection (44 fmol), 210 nm at 70 cm. (A) 1 kPa (10 mbar) on inlet vial, (B) no pressure on inlet vial.

observed, nor was the efficiency improved. Only the elution time was slightly reduced.

When using the CElect-Amine columns in conjunction with the liquid sheath source, we still applied a 1 kPa pressure in order to eventually eliminate penetration or diffusion of the liquid sheath solution in the separation capillary. The liquid sheath flow was set to a low value, 0.5–0.7 μl min^{-1}, in order to avoid deleterious effects on signal intensity or separation efficiency. However it had to be sufficiently high to ensure spray stability. Increasing the liquid sheath flow from 0.5 to 2 μl min^{-1} resulted in significant reduction of the signal intensity (Fig. 5), and in our experiment this effect was stronger than reported by other groups [29]. We believe that this strong signal reduction with increase in liquid sheath flow is also related to the ability of the TOF ion source and sampling arrangement to dry, or decluster, the ions. At the very beginning of our experiments, after the mass spectrometer became functional, we experienced a similar effect with the microspray source when it was operated at higher than 0.5 μl min^{-1} or was positioned very close to the nozzle orifice. Ion currents could be measured along the ion path in the mass spectrometer even beyond the pulsing region because of the penetration of the droplets, however, there was no signal at the detector. Some of our previous experiments performed with the liquid sheath source and a Sensar TOF 1000 mass spectrometer (Sensar, Provo, UT, USA) did not reveal such a strong effect on signal intensity, or even signal loss.

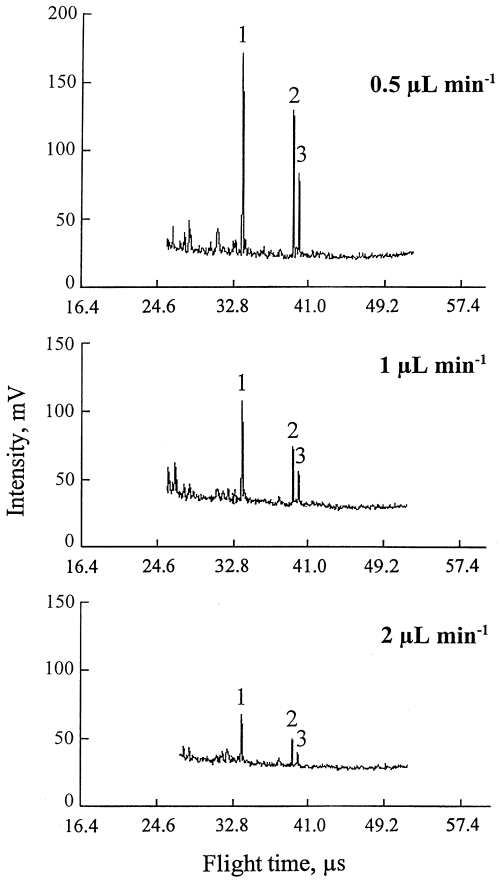

Fig. 5. Effect of liquid sheath flow-rate on signal intensity for enkephalins. Conditions: CE infusion through 90 cm×50 μm I.D. capillary, 30 kV, 1 kPa. Liquid sheath: methanol–water–acetic acid (80:20:0.1, v/v), ESI (2650 V), 70°C. Peak identifications: (1) des-Tyr[1],[D-Ala[2], D-Leu[5]]-enkephalin, (2) leucine enkephalin, (3) methionine enkephalin [134–188 μ*M* in methanol–water–acetic acid (50:50:0.03, v/v)].

3.4. Gas flows

The curtain gas, which assists in the desolvation of droplets, was absolutely necessary. It was usually set at 1200 ml min^{-1} and 40–90°C. Only when using the microspray and operating at very low flow-rates (less than 0.3 μl min^{-1}) were we able to obtain a reasonably good signal without using the curtain gas.

Nebulizing gas turned out not to be necessary at the given liquid flow-rates of the liquid sheath electrospray. A low flow of sulfur hexafluoride make-up gas (~200 ml min^{-1}) proved to be useful when purely aqueous solutions were sprayed to suppress a small discharge present at the spray tip.

3.5. Ion source temperature

It was necessary to heat the ion source when the liquid sheath source was used, when the microspray was positioned close to the nozzle, or when it was operated at higher than 0.5 μl min^{-1}. Although for most applications the temperature of the source (actually the temperature of the nitrogen counter current gas) was set at 60°C, it was sometimes necessary to use 90°C.

3.6. CE–MS interfacing

The above described microelectrospray source proved to be a very simple and reliable tool for continuous infusion experiments. For CE–MS, we reported very good detection levels with this set-up (1 fmol injected) [24], however interfacing was not easy to perform. Some of these electrospray tips behaved well, while others did not. Some of the perturbing phenomena included: interrupted or fluctuating flow and ESI signal, gas bubbles produced at the tip and suppressed analyte elution, probably due to the loss of electrical contact at the spray tip. The cracked capillaries induced no loss of efficiency or resolution, while the cut capillaries performed well only when the alignment between the tip and separation capillary was perfect. A comparison between the electropherograms obtained with CE–UV (approximately 200 000 plates) and CE–TOF (approximately 150 000 plates) using the microelectrospray source is given in Fig. 6. The loss of efficiency is due to the cut capillary. A slightly larger quantity was injected for the CE–UV run (44 fmol from a 40 μ*M* solution) than for the CE–TOF run (11 fmol from a 10 μ*M* solution), which resulted in a larger signal.

An evaluation of the liquid sheath effect with respect to preservation of the CE separation efficiency for a mixture of enkephalins was conducted using commercially available CElect-Amine columns (Fig. 7), which produced much more efficient sepa-

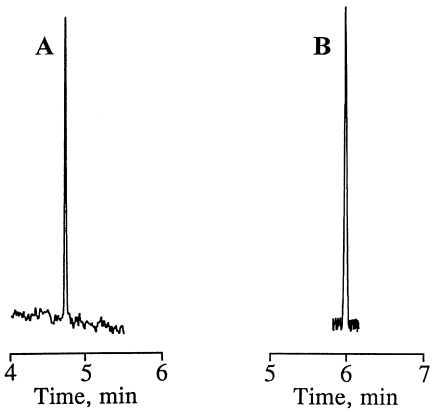

Fig. 6. CE–UV and CE–TOF-MS electropherogram of leucine enkephalin. Conditions: 85 cm×50 μm I.D. uncoated fused-silica capillary, acetonitrile–water–acetic acid (50:50:0.3, v/v), 30 kV, 1 kPa, 0.3 μA, 2 kPa×0.05 min injection. (A) CE–UV: 40 μ*M* solution, 210 nm at 70 cm. (B) CE–TOF: 10 μ*M* solution, μESI (1650 V), 80°C. MS data acquisition: 5000 Hz pulsing rate, 1000 spectra averaged, 5 data points s^{-1}.

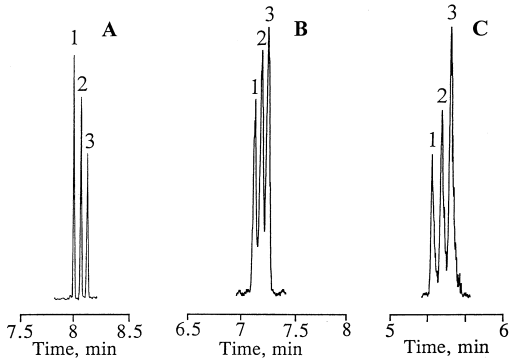

Fig. 7. CE–UV and CE–TOF-MS separation of enkephalins. Conditions: 90 cm×50 μm I.D. amine-coated fused-silica, 0.3% acetic acid in water, 1 kPa, 3 kPa×0.3 min injection (~1.2–1.7 pmol from a 133–188 μ*M* solution). (A) CE–UV: −25 kV, −278 V cm^{-1}, −2.1 μA, 210 nm at 75 cm. (B) CE–TOF: −25 kV, −313 V cm^{-1}, −2.4 μA. Liquid sheath: 0.5 μl min^{-1}, methanol–water–acetic acid (80:20:0.1, v/v), ESI (3175 V), 90°C. MS data acquisition: 10 000 Hz pulsing rate, 2000 spectra averaged, 5 data points s^{-1}. (C) CE–TOF: conditions are the same as in (B), except: 68 cm×50 μm I.D. amine-coated fused-silica, −408 V cm^{-1}, −3.5 μA, ESI (2750 V). MS data acquisition: 5000 Hz pulsing rate, 1000 spectra averaged, 5 data points s^{-1}. Peak identifications: (1) methionine enkephalin acetate, (2) leucine enkephalin acetate, (3) des-Tyr[1],[D-Ala[2], D-Leu[5]]-enkephalin acetate.

rations than the bare fused-silica columns. The experimental conditions are given in the legend. The peaks are baseline separated in the CE–UV electropherogram, but some loss of resolution is observed in the CE–TOF-MS electropherogram. The efficiency dropped from 370 000–460 000 plates in the CE–UV separation to 230 000–260 000 in the CE–TOF-MS separation. This effect is probably due to the liquid sheath. In most cases, we operated the liquid sheath at 0.5 μl min^{-1}, the minimum value at which we still obtained a reasonably stable spray for a long period of time. The EOF was estimated to be 220 nl min^{-1}. The flow of the liquid sheath and the relative position of the CE capillary and spray needle tip have an important influence on the preservation of separation efficiency and resolution, and obviously the effect is much greater for fast and narrow eluting peaks. The three enkephalins eluted within 15–17 s and had base-peak widths of about 3–4 s in the CE–UV experiment. Reproducing the same separation on a different day, on a shorter capillary (68 cm), and under the same conditions, resulted in a less significant loss of resolution (Fig. 7C). An acquisition rate of 5 data points s^{-1} (5000/10 000 Hz pulsing rate, 1000/2000 averaged spectra per data point) proved to be sufficient for reliable monitoring of these separations. The peaks were defined by 20–30 data points. In order to reduce the amount of stored data, data collection usually started 30–40 s prior to the elution of analytes. The ESI source produced protonated molecular ions for these analytes. The electropherograms were re-constructed by integrating each mass spectrum within the mass range of interest.

We experienced extremely reproducible separations with the CElect-Amine columns when using the UV detector. When the CE was interfaced to the TOF-MS system, we observed a significant increase in migration times (almost two times) on some of the separation columns after several injections were performed. This is much more than would be expected if we consider that UV detection occurred at 75 cm, on the 90 cm column, while TOF-MS detection occurred at the end of the column. At the present time we do not have a reasonable explanation for this effect. Penetration of the methanolic liquid sheath flow inside the CE capillary, despite the 1 kPa applied pressure at the inlet, would induce such behavior. The deterioration of the modified inner surface of the capillary inside the heated ion source might also be a contributing factor. Further studies are necessary to completely clarify these results. However, in the case of the separation in Fig. 7B, the elution time actually decreased. The increase in field strength in the separation capillary during the CE–TOF separation, due to the contribution of the ESI voltage may be, at least partially, responsible for the reduction in migration times.

To obtain a strong signal with the UV detector, a relatively large amount of analytes were injected on the column (i.e., 1.2–1.7 pmol from a 133–188 μ*M* solution). Using the TOF-MS system, we observed a very good signal even at approximately ten-times lower amount (i.e., 130–180 fmol from a 13–18 μ*M* solution). When this experiment was performed, the compounds had much higher migration times than expected, and the measured efficiencies were lower (i.e., 25 000–40 000 plates, Fig. 8).

Similar behavior was observed for the separation of five peptides (Fig. 9). Peaks 1 and 2 are baseline resolved in both CE–UV and CE–TOF runs. The different ratios of peak heights in the CE–UV and CE–TOF separations, for both mixtures, enkephalins and peptides, are due to the fact that some of the analytes contain stronger UV-absorbing residues than others.

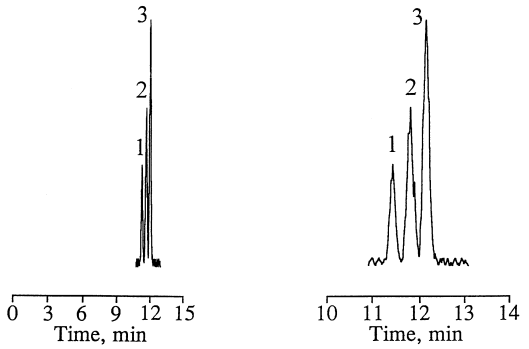

Fig. 8. CE–TOF-MS separation of enkephalins. Conditions: 82 cm×50 μm I.D. amine-coated fused-silica, 0.3% acetic acid in water, −25 kV, −340 V cm^{-1}, −2.7 μA, 1 kPa, 3 kPa×0.3 min injection (~130–180 fmol from a 13–18 μ*M* solution). Liquid sheath: 0.7 μl min^{-1}, methanol–water–acetic acid (80:20:0.1, v/v), ESI (2950 V), 90°C. MS data acquisition: 5000 Hz, 5000 spectra averaged, 1 data point s^{-1}. Peak identifications: (1) methionine enkephalin acetate, (2) leucine enkephalin acetate, (3) des-Tyr[1],[D-Ala[2], D-Leu[5]]-enkephalin acetate.

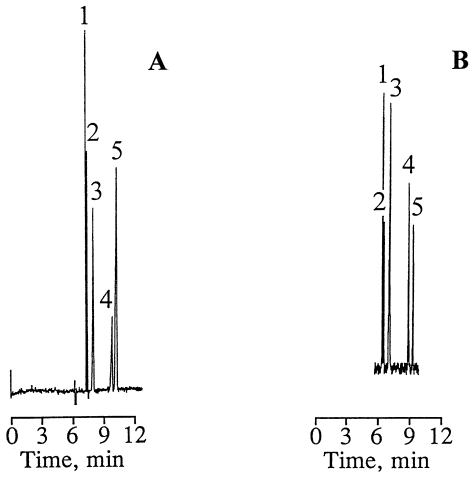

Fig. 9. CE–UV and CE–TOF-MS separation of peptides. (A) CE–UV: 90 cm×50 μm I.D. amine-coated fused-silica, 0.3% acetic acid in water, −25 kV, −278 V cm^{-1}, −2.1 μA, 1 kPa, 3 kPa×0.3 min injection (~1–1.2 pmol from a 110–130 μ*M* solution), 210 nm at 75 cm. (B) CE–TOF: 75 cm×50 μm I.D. amine-coated fused-silica, 0.3% acetic acid in water, −25 kV, −369 V cm^{-1}, −2.8 μA, 1 kPa, 3 kPa×0.15 min injection (~600–700 fmol from a 110–130 μ*M* solution). Liquid sheath: 0.5 μl min^{-1}, methanol–water–acetic acid (80:20:0.1, v/v), ESI (2700 V), 90°C. MS data acquisition: 5000 Hz, 5000 spectra averaged, 1 data point s^{-1}. Peak identifications: (1) Phe–Gly–Gly–Phe, (2) Ala–Gly–Ser–Glu, (3) Val–Pro–Leu, (4) Arg–Gly–Asp, (5) Gly–Gly–Tyr–Arg.

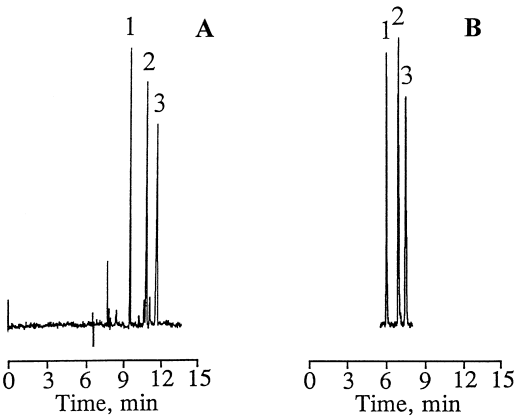

Fig. 10. CE–UV and CE–TOF-MS separation of angiotensins. (A) CE–UV: 90 cm×50 μm I.D. amine-coated fused-silica, 0.3% acetic acid in water, −25 kV, −278 V cm^{-1}, −2.1 μA, 1 kPa, 3 kPa×0.3 min injection (~500–700 fmol), 210 nm at 75 cm. (B) CE–TOF: 68 cm×50 μm I.D. amine-coated fused-silica, 0.3% acetic acid in water, −25 kV, −408 V cm^{-1}, −3.5 μA, 1 kPa, 3 kPa×0.15 min injection (~660–920 fmol). Liquid sheath: 0.5 μl min^{-1}, methanol–water–acetic acid (80:20:0.1, v/v), ESI (2730 V), 90°C. MS data acquisition: 5000 Hz, 5000 spectra averaged, 1 data point s^{-1}. Peak identifications: (1) angiotensin II, (2) angiotensin I, (3) angiotensin III.

The liquid sheath had less visible effect when broader peaks were obtained. For the separation of some angiotensins (Fig. 10), the ESI source mainly produced the doubly protonated molecular ions for angiotensins II (1046 u) and III (931 u), and the triply protonated molecular ion for angiotensin I (1296 u). Shorter columns have been used for the CE–TOF separation of peptides and angiotensins in order to reduce the migration times.

4. Conclusions

An electrospray source was built and evaluated for interfacing CE to TOF-MS. The main contributing factors to the spray stability were observed and optimized. The performance of the electrospray source was evaluated with respect to preservation of separation efficiency for CE. It was especially important for very efficient separations that the effect of the ionization source necessitated careful study. There are numerous papers which deal with interfacing of CE to MS, however, this question has not been fully answered. It was observed in our experiments that the liquid sheath electrospray source produced up to 50% drop in efficiency for peaks originally giving as high as 460 000 theoretical plates. For the case of less efficient, more typical separations, the liquid sheath was less detrimental. The loss of efficiency and resolution was very much dependent on the liquid sheath flow and the position of the CE capillary relative to the spray needle. The microspray configuration allowed operation without application of a gold coating on the spray tip, however, if the set-up was not correct, an efficiency loss of up to 25% occurred. Both liquid sheath and microspray configurations allowed determination of analytes in the low fmol range.

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