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Design and performance of a sheathless capillary electrophoresis/mass spectrometry interface by combining fused-silica capillaries with gold-coated nanoelectrospray tips

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A simple sheathless capillary electrophoresis (CE)/mass spectrometry (MS) interface was constructed by combining widely used nanospray needles with fused-silica capillaries and it was successfully applied for the separation of peptides. The end of the CE capillary was pulled to a taper, etched and then fitted into the metal-coated nanospray borosilicate capillary. The nanospray needle can be used for several CE runs, but it can be easily and rapidly changed in the case of accidental breakage or evaporation of the coating. A fast capillary electrochromatographic method was also developed for MS analysis of peptides containing numerous basic amino acids.

The coupling of mass spectrometry (MS) with liquid-phase separation systems has great potential because it provides efficient separation and also selective mass identification. Capillary electrophoresis (CE) is a fast, highly efficient separation method for small sample quantities and, interfaced with electrospray ionization (ESI) MS, it has become one of the most powerful techniques for the separation and selective mass identification of charged analytes.[1]

One of the major challenges to the use of ESI-MS as an online detector for CE is the provision of a closed circuit for both the CE separation and the ESI process. The designs used to accomplish this electrical connection can be divided in three general methods: sheath flow,[2] liquid junction[3] and sheathless interfaces.[4]

In the case of the sheath flow interface coaxially pumped sheath liquid is used to provide the electrical contact with the capillary outlet. This continuous liquid stream improves electrospray stability and allows operation over a wide range of buffer conditions. The relatively high sheath liquid flow also has disadvantages, not only due to the considerable dilution of the analytes by the sheath liquid, degrading the overall sensitivity, but also because the nonoptimal electrospray conditions result in decreased ionization efficiency. The sensitivity of the measurement is significantly increased by decreasing the i.d. at the tapered electrospray tip; therefore, nanospray sheath flow interfaces were developed. In this instance the separation capillary was joined to a tapered borosilicate capillary containing a platinum electrode to connect the high voltage. In this arrangement the sheath flow was fed through a second fused-silica capillary or through the borosilicate capillary.[5,6]

In the case of the liquid junction interface,[3] the electrospray voltage is applied to a liquid, which fills the space between two end-to-end butted capillaries. This interface requires precise alignment of the separation capillary and the electrospray tip. Poor alignment results in a large dead volume at the junction causing low sensitivity and separation efficiency.

The sheathless interface requires no make-up buffer and therefore the analytes are not diluted. Thus, the sample is sprayed directly into the inlet of the mass spectrometer and the concentration is higher than for the sheath flow interface, hence the ionization efficiency and the sensitivity are higher as well. A high diversity of designs has been described both for providing electrical potential to the electrospray tip and for closing the electrical circuit; for example: (1) metal,[7–9] graphite[10–12] or conductive polymer coating of the tapered end of the capillary;[13] (2) plugging a metal wire into the outlet or through a hole in the wall of the capillary;[14] (3) a spray tip attached to the capillary outlet in different ways (micro tee, tubing made from stainless steel or porous glass, microdialysis tubing[15]); (4) split flow interface where the wall of the capillary is punched and then coaxially inserted into a metal sheath closing the circuit through the split flow;[16] (5) production of a porous, conductive wall on the separation capillary by etching with hydrogen fluoride.[17]

In principle the single-piece sheathless interfaces are a preferable choice for interfacing CE to MS compared with the end-to-end butted disposable emitter tips, but in particular cases the latter tips can offer a better alternative. If the

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fabrication of the electrical connection is preceded by a time-consuming, tedious pretreatment, as in the case of the coated or capillary electrochromatography (CEC) columns, it is practical to avoid additional risks of damaging fragile capillary tips. While a damaged bare capillary can be replaced easily by a new one, for the capillary columns requiring a long preparation procedure, the disposable emitter tips seem to be a better alternative.

In this work, a simple design of a sheathless interface was constructed and used for the separation of peptides by combining the widely used nanospray gold-coated borosilicate tips and the separation capillary. The nanospray needle fabricated in our laboratory, but also available commercially, can be used for several runs. It can be changed easily and rapidly, and therefore it was successfully used for CEC columns as well. Owing to the low flow rate of the nanospray emitters, stable electrospray can be obtained under acidic conditions when the endoosmotic flow (EOF) is suppressed. This device can be easily prepared in every laboratory where the mass spectrometer is equipped with a nanospray ion source.

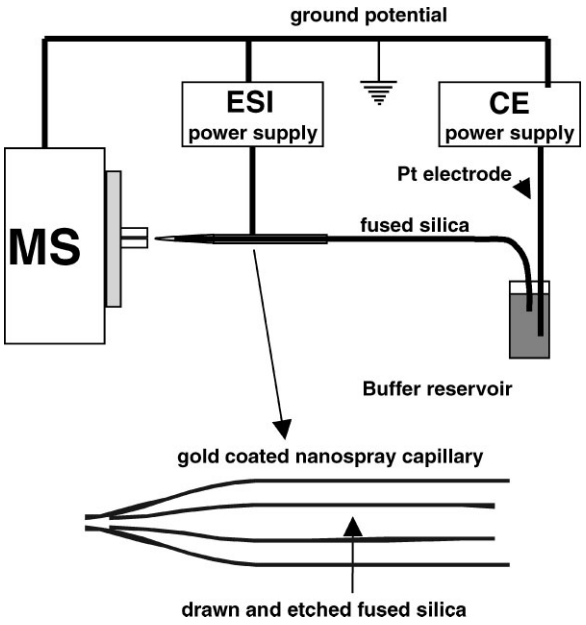

Figure 1. The CE/ESI-MS interface.

EXPERIMENTAL

Apparatus

All experiments were performed using a Finnigan TSQ 7000 triple quadrupole instrument (Finnigan MAT Ltd, San Jose, CA, USA) equipped with a Finnigan electrospray source and with a home-built nanospray ion source.[18]

The nanospray tips were obtained by pulling to a taper borosilicate glass capillaries (1.2 mm o.d., 0.69 mm i.d.; Clark Electromedical Instruments, Reading, UK) that had previously been sealed at both ends by heating with a microtorch in order to avoid contamination during the subsequent procedure. The capillaries were pulled with a microcapillary puller (Sutter Instrument Co., Novato, CA, USA) in such a way that the capillaries ended in a very fine glass filament. A thin layer of gold was deposited on the full outer surface of the needles using a vapor deposition instrument (Edwards, Crawley, UK).

Fused-silica capillaries (i.d. 50 μm, o.d. 150 μm; Polymicro Technologies, Phoenix, AZ, USA) were used to fabricate the interface according to the following procedure. A vertically suspended section of capillary, to which a small weight (about 30 g) had been attached, was drawn to a taper using a propane-butane microtorch. The tapered tip was etched in 48% aqueous hydrogen fluoride solution for 10 min after the other end of the capillary had been immersed in water, in order to prevent ingress of the acid by capillary action. After this procedure the capillaries were conditioned with 1 M NaOH, washed with deionized water by sucking with a water-jet pump, and then cut into 20 cm length.

For preparing CEC capillaries a simple slurry packing method was used. A plastic syringe was filled with slurry of perfusion chromatographic media (POROS R2-10; Perseptive Biosystems, Foster City, CA, USA) and then the syringe was attached to the end of the fused-silica capillary. When the slurry was pushed through the capillary the packing material was retained by the tapered end and therefore no frit was used (15 cm length of the capillary was packed).

The completed capillary was filled with running buffer using a Hamilton glass syringe via a Teflon connector. The nanospray capillary tip was adjusted to the proper position on the stage of the interface for electrospraying. The dimensions of the tips were determined with a binocular microscope (Olympus IX 71). The tapered tip of the separation capillary (5–7 μm i.d., 15–20 μm o.d. at the end of the tips) was inserted coaxially into the borosilicate tip, ensuring a tight fit to the tapered end of the borosilicate (4–6 μm i.d. at the end of the tips). The inner capillary was fixed with a rubber block, which was forced down by an O-ring to the stage of the interface. The distance between the separation capillary tip and the nanospray tip was 40–60 μm. The opposite end of the fused-silica capillary was immersed into the running buffer. The CE high-voltage power supply (Spellman, Plainview, NY, USA) was connected to a platinum electrode, which was inserted into the same Eppendorf tube. The ESI high-voltage power supply was connected directly to the gold-coated surface of the borosilicate capillary (Fig. 1). Voltages of 1.3 kV (nanospray needle) and 15 kV (CE capillary inlet) were applied in these experiments.

Materials

A mixture of ethanol/water/acetic acid (20:79:1 v/v/v) was used as solvent in all experiments. Perfusion chromatographic media (POROS R2-10) were from Perseptive Biosystems. Peptides: H-RIIGL-NH_2, propionyl-RIIGL-NH_2, H-RGAGGLGLGK-NH_2, Ac-RGGGGLGLGK-NH_2, Ac-RGAGGLGLGK-NH_2, Ac-RGVGGLGLGK-NH_2, and VEPKVKKREAVAGRGRGRGRGRGRGRGRGGPRR (0.01 mg/mL, dissolved in the running buffer), were synthesized in our laboratory.

RESULTS AND DISCUSSION

One of the prerequisites for successful CE/MS experiments is a reliable and stable electrical contact at the capillary outlet. In

this interface (Fig. 1), the high-voltage power supply of the ESI source was connected to the gold-coated outer surface of the borosilicate capillary. The injection end of the fused-silica capillary, pre-filled with buffer solution, was immersed into the buffer reservoir and the tapered end was inserted into the nanospray tip. A small volume of buffer was pulled into the tip of the borosilicate capillary by capillary action filling the truncated cone shaped cave (about 0.004 nL, calculated using the dimensions measured with binocular microscope) between the two tips. This liquid closed the electrical circuit, as could be followed by measuring the intensity of the current produced by the CE power supply.

Inserting the tapered separation capillary into the borosilicate tip is the crucial point of the design. In order to make the insertion easy the separation capillary was guided through a plastic pipette tip fitted to the end of the previously fixed nanospray capillary and into the nanospray tip. Gentle bending of the separation capillary indicated that the end of the nanospray tip had been reached. This was the reason why the more flexible 150 μm o.d. capillary was chosen instead of the customary 360 μm o.d. capillary. The flexibility of the smaller diameter capillary helps to protect the fine tip of the nanospray needle against breaking when it is fitted to the nanospray tip. The procedure can be followed by stereomicroscopy due to the transparency of the thin layer of gold and thus any accidental breakage of the nanospray tip can be seen.

Another prerequisite of the coupling procedure is to obtain stable electrospray at CE flow rates. It is known that stable electrospray can be achieved using metal-coated borosilicate capillaries for long duration at low flow rates.[19] Even lower flow rates were used in our experiments due to acidic conditions resulting in suppression of the EOF. The stability of the system was tested by setting the same conditions that were intended to be used for separation of peptides. It was found that the electrospray signal and the CE current were stable for 45 min (maximum studied duration), which was sufficient time for CE/MS analysis.

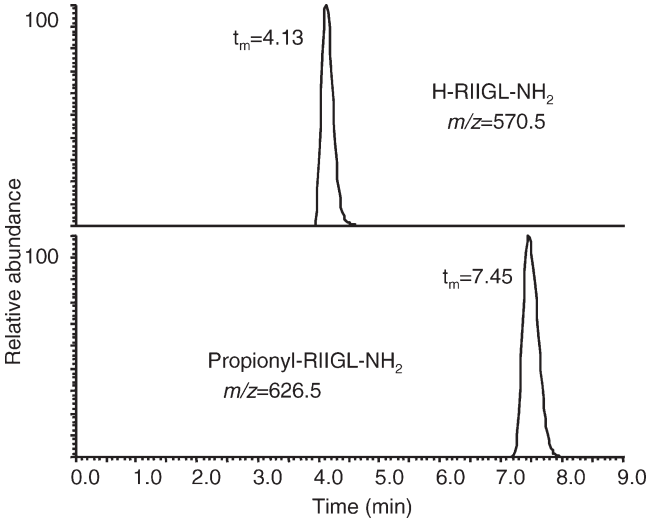

Figure 2. Mass electrophorograms of protonated H-RIIGL-NH_2 and propionyl-RIIGL-NH_2 peptides (length of the capillary: 20 cm; running buffer: methanol/water/acetic acid 20:79:1 v/v/v; −15 kV to the injection end of the column and −1.3 kV to the nanospray needle were applied; electrokinetical injection: −5 kV, 2–3 s).

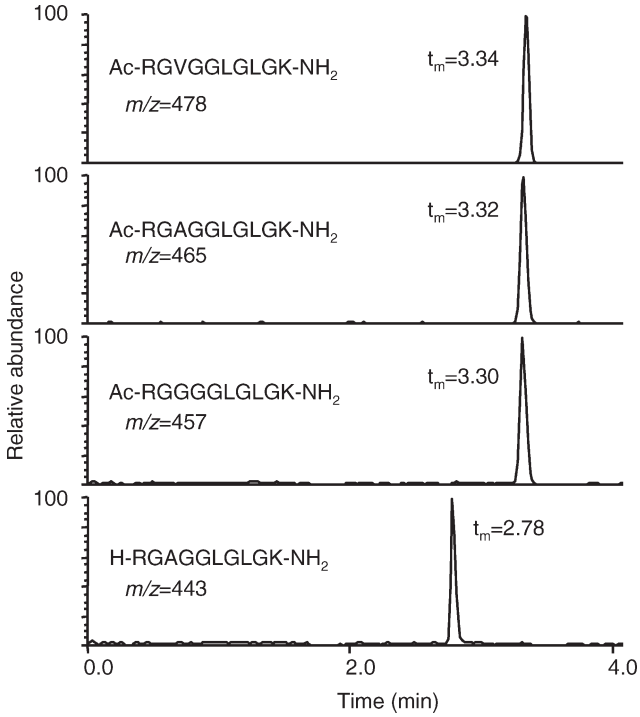

Figure 3. Mass electrophorograms of the doubly protonated ions of the mixture of four peptides (for conditions, see Fig. 2).

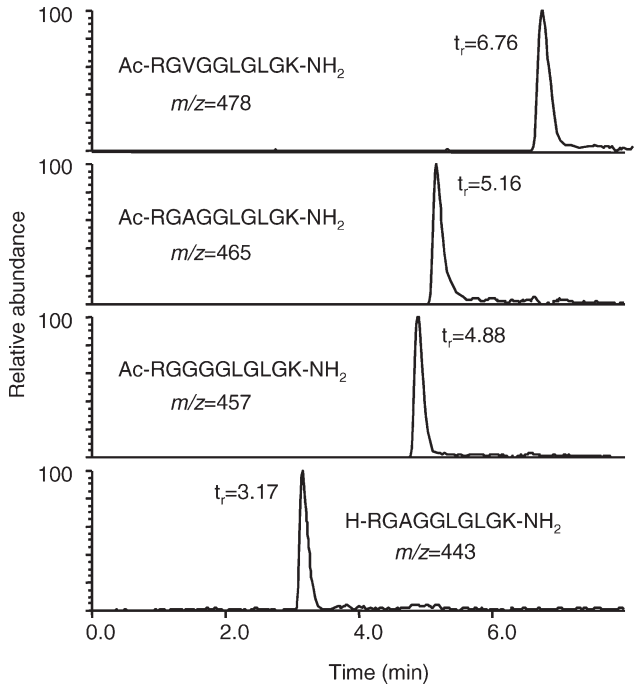

Figure 4. Mass electrochromatograms of the doubly protonated ions of the mixture of four peptides (length of the capillary: 20 cm; packed section of the capillary: 15 cm; perfusion chromatographic media: POROS R2-10; for further conditions, see Fig. 2).

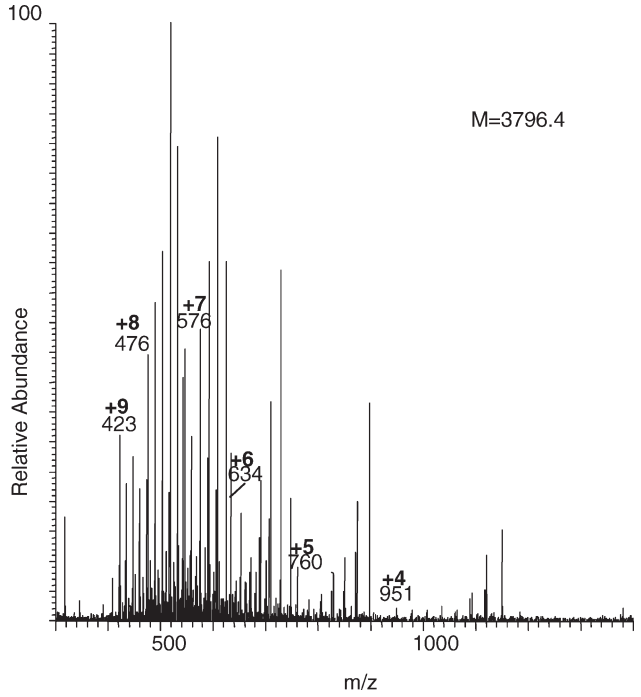

Figure 5. ESI-MS analysis of the cationic peptide VEPKVKKREAVAGRGRGRGRGRGRGRGRGGPRR, which was dissolved in methanol/water/acetic acid (20:79:1 v/v/v). The same solvent was also used for electrospraying.

To evaluate the CE/MS interface design a mixture of two peptides (H-RIIGL-NH_2 and propionyl-RIIGL-NH_2) was electrokinetically injected by applying a voltage of −5 kV (duration: 2–3 s) to the electrode in the sample reservoir. Figure 2 shows the mass electrophorograms of the aforementioned two peptides. Components were detected within 8 min and, as expected (due to the N-terminal protection the second peptide has only one basic function), the two peptides were separated successfully. Another mixture of peptides (H-RGAGGLGLGK-NH_2, Ac-RGG-GGLGLGK-NH_2, Ac-RGAGGLGLGK-NH_2, Ac-RGVGG-LGLGK-NH_2) with similar structure was used to evaluate the CEC/MS coupling. This sample is routinely employed in our laboratory to test the resolving power of analytical high-performance liquid chromatography (HPLC) columns. Using CE/MS, baseline separation of these components was not possible, except for the first component with a free N-terminal amino group (Fig. 3). With the slurry-packed CEC capillary all four peptides were successfully separated (Fig. 4).

This arrangement can also be used in cases which do not require separation but where the analysis is difficult using electrospray or nanoelectrospray. The peptide VEPKVKK-REAVAGRGRGRGRGRGRGRGRGGPRR (previously purified using a water/acetonitrile/trifluoroacetic acid (TFA) gradient) contains numerous basic amino acids which easily form salts with TFA. Figures 5 and 6 show the electrospray and the nanospray spectra of the peptide (M = 3796.4) under acidic conditions, respectively. The spectrum in Fig. 5 contains the corresponding low abundance, multiply charged ions ($z = +9, 8, 7, 6, 5$; m/z =423, 476, 576, 634, 760) together with several abundant, confusing peaks. The nanospray spectrum (Fig. 6) can be more easily interpreted than that produced by electrospray but further series of ions are observable in the neighborhood of every expected ion, mostly at higher m/z values. These series can be assigned to TFA salts of the peptide with different TFA content. For example, the series of doubly charged ions (Fig. 6, inset) correspond to the masses of the different ion pairs formed between the peptide and TFA. The difference between the ions in the doubly charged series is 56.5 Th, half of the mass of the trifluoroacetate ion. This peptide is positively charged in acidic conditions and therefore a short elution time was expected in the case of CEC analysis. Figure 7

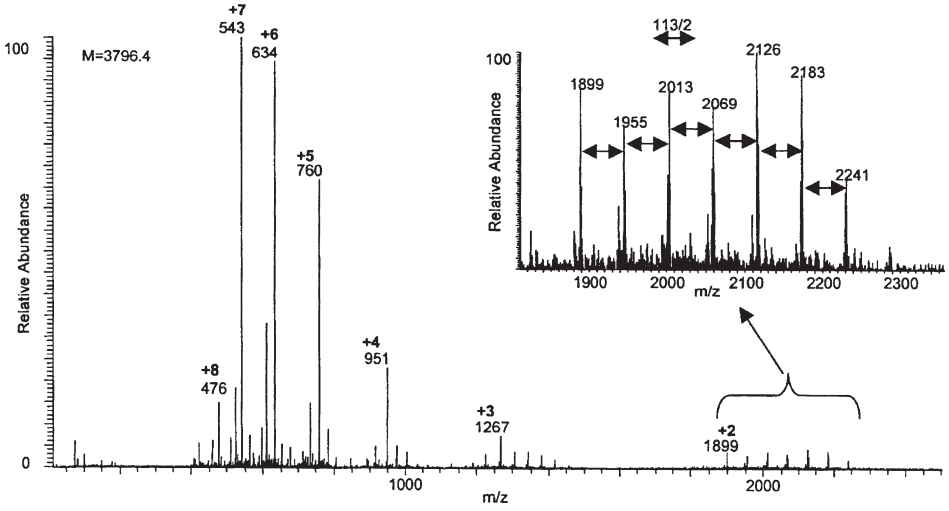

Figure 6. Nano-ESI-MS analysis of the cationic peptide VEPKVKKREAVAGRGRGRG-RGRGRGRGRGRGGPRR, which was dissolved in methanol/water/acetic acid (20:79:1 v/v/v). This mixture was also used for electrospraying. The magnified part of the spectrum shows the doubly charged ions in the spectrum. The mass difference between the peaks is 56.5 Th, corresponding to half of the mass of the trifluoroacetate ion.

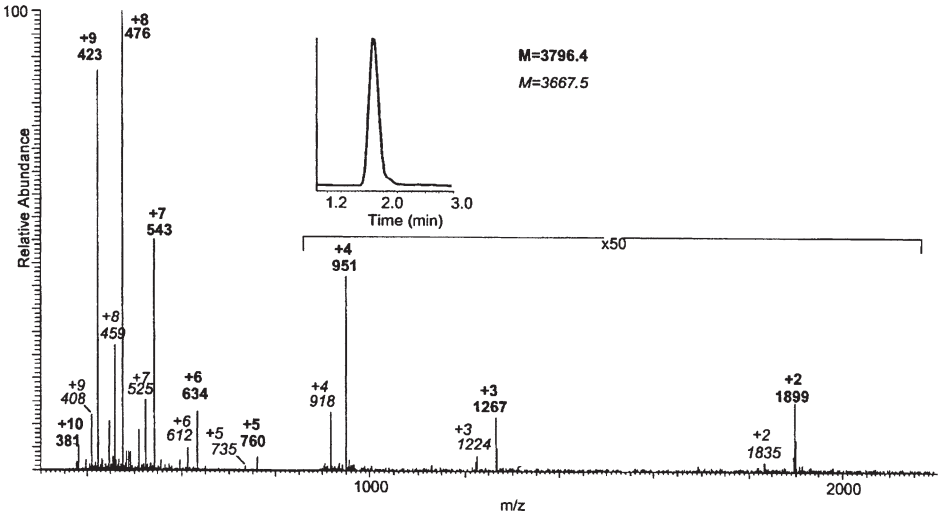

Figure 7. CEC-ESI-MS analysis of the cationic peptide VEPKVKKREAVAGRGRGRG-RGRGRGRGRGRGGPRR, which was dissolved in methanol/water/acetic acid (20:79:1 v/v/v). This mixture was also used as eluent. The inset shows the capillary electrochromatogram of the peptide. The ion series corresponding to the peptide is indicated in bold numbers. Ion series labeled in italics belong to a deletion sequence (-Lys) of the peptide. For further conditions, see Fig. 4.

shows the mass spectrum and the capillary electrochromatogram of the peptide with a retention time of less than 2 min (see the inserted electrochromatogram). The ions originating from the TFA salts disappeared and thus the spectrum can be interpreted more easily. Ions at lower *m/z* values are due to a deletion sequence of the peptide (–Lys, M = 3667.5).

CONCLUSIONS

A cheap and simple sheathless interface has been developed and used for CE and CEC separation of peptides. The design is based on an existing nanoelectrospray ion source and therefore it can be coupled easily to any mass spectrometer equipped with this ion source. In the case of accidental decomposition of the gold surface, the disposable nanospray tips can be rapidly changed. These tapered borosilicate tips provide a stable electrospray signal at the low flow rates of CE under acidic conditions, when the EOF is suppressed. This arrangement does not require an inner electrode, because the electrical contact of the outlet of the separation capillary is provided through the metal coating of the nanospray tip. Thus the experiments are not hampered by the formation of bubbles which would occur on the surface of an inner electrode.

This CE/CEC method can also be used as an alternative to direct nanoelectrospray analysis when the purity of the sample does not allow successful direct analysis. A fast CEC method has also been described for the mass spectrometric analysis of peptides containing numerous basic amino acids where interfering ions from TFA salts were successfully removed while the time of analysis remained within a few minutes.

Acknowledgements

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REFERENCES

1. Schmitt-Kopplin P, Frommberger M. *Electrophoresis* 2003; **24**: 3837.
2. Smith RD, Barinaga CJ, Udseth HR. *Anal. Chem.* 1988; **60**: 1948.
3. Lee ED, Muck W, Henion JD, Covey TR. *Biomed. Environ. Mass Spectrom.* 1989; **18**: 844.
4. Olivares JA, Nguyen NT, Yonker CR, Smith RD. *Anal. Chem.* 1987; **59**: 1230.
5. Hsieh F, Baronas E, Muir C, Martin SA. *Rapid Commun. Mass Spectrom.* 1999; **13**: 67.
6. Chen YR, Tseng MC, Chang YZ, Her GR. *Anal. Chem.* 2003; **75**: 503.
7. Bendahl L, Hansen SH, Olsen J. *Rapid Commun. Mass Spectrom.* 2002; **16**: 2333.
8. Kriger MS, Cook KD, Ramsey RS. *Anal. Chem.* 1995; **67**: 385.
9. Chen YR, Her GR. *Rapid Commun. Mass Spectrom.* 2003; **17**: 437.
10. Nilsson S, Wetterhall M, Bergquist J, Nyholm L, Markides KE. *Rapid Commun. Mass Spectrom.* 2001; **15**: 1997.
11. Chang YZ, Her GR. *Anal. Chem.* 2000; **72**: 626.
12. Chang YZ, Chen YR, Her GR. *Anal. Chem.* 2001; **73**: 5083.
13. Wetterhall M, Nilsson S, Markides KE, Bergquist J. *Anal. Chem.* 2002; **74**: 239.
14. Cao P, Moini M. *J. Am. Soc. Mass Spectrom.* 1997; **8**: 561.
15. Severs JC, Harms AC, Smith RD. *Rapid Commun. Mass Spectrom.* 1996; **10**: 1175.
16. Moini M. *Anal. Chem.* 2001; **73**: 3497.
17. Janini GM, Conrads TP, Wilkens KL, Issaq HJ, Veenstra TD. *Anal. Chem.* 2003; **75**: 1615.
18. Kele Z, Janáky T, Mészáros T, Fehér A, Dudits D, Szabó PT. *Rapid Commun. Mass Spectrom.* 1998; **12**: 1564.
19. Wilm M, Mann M. *Anal. Chem.* 1996; **68**: 1.