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Capillary electrophoresis with electrospray mass spectrometry detection for low-molecular-mass compounds

Thomas E. Wheat[a,*], Kim A. Lilley[a], J. Fred Banks[b]

[a]*Thermo CE, 8 East Forge Parkway, Franklin, MA 02038, USA*

[b]*Analytica of Branford, Inc., 29 Business Park Drive, Branford, CT 06405, USA*

Abstract

Mass spectrometry (MS) detection using electrospray ionization (ESI) has been explored for the separation by capillary electrophoresis (CE) of a number of sample mixtures containing low-molecular-mass species. Optimal sheath liquid composition has been determined using a peptide mixture in which femtomolar quantities of analyte were easily observed. Effects of CE buffer choice were studied in detail. Also, a separation of basic drugs in cough syrup has been successfully detected by ESI–MS. Using negative ionization, a mixture of alkyl sulfonates and a mixture of food dyes were analyzed. All components were easily resolved and identified by molecular weight. © 1997 Elsevier Science B.V.

Keywords: Capillary electrophoresis–mass spectrometry; Electrospray ionization; Interfaces; Detection, electrophoresis; Peptides

1. Introduction

The direct coupling of capillary electrophoresis (CE) with mass spectrometry (MS) provides a more useful analytical tool than either technique alone. Separation techniques, in general, are useful for improving the sensitivity of mass spectrometry and for simplifying interpretation of the analytical results. The chemical selectivity of CE separations is different from that of HPLC and GC, the other techniques commonly used with MS. Furthermore, CE gives good resolution and high sensitivity because of its intrinsically high plate counts, and is also generally more tolerant of complex samples. Mass spectral data enhance the utility of CE by providing structural information for assessment of peak purity and identity.

Electrospray ionization (ESI) is the method of choice interfacing CE and MS. It both ionizes and desolvates the analytes of interest, providing good yields of the molecular ion for high sensitivity with polar, fragile and thermally labile analytes. CE–ESI has been coupled to a variety of MS techniques including quadrupole [1–3], ion trap [4,5], magnetic sector [6], Fourier transform ion cyclotron resonance [7], and time-of-flight [8,9]. In all these examples, it was necessary to solve a variety of electrical, physical, and chemical problems.

The most significant of these problems arises because the CE column is composed of fused silica, a dielectric that prevents grounding of the CE circuit and cannot be incorporated into the ESI system. This electrical problem was originally addressed with the use of a coaxial sheath liquid [2]. Numerous alternative designs have been developed to improve sensitivity [7,8,10–12], but the sheath design remains the most reliable and easiest to implement. The liquid both grounds the CE circuit and satisfies

*Corresponding author.

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the ESI conditions. To eliminate any electrical connection between the ESI and CE power supply, most commercial CE manufacturers now make provision for a floating ground and internal loopback in the power supply or offer additional hardware providing these functions. With these solutions to the physical and electrical aspects of the interface, chemical issues can be addressed in detail.

The sheath liquid not only provides electrical contact between the separation capillary and the ESI needle, but also provides the bulk of the liquid for the electrospray process. As with any CE technique, the running buffer determines the quality of the separation. Both liquids affect the transfer of analyte ions from the liquid phase into the gas phase. In general, these requirements imply the use of low concentrations of volatile buffers in both, usually at low pH, with a largely organic sheath liquid. Furthermore, the sheath liquid is essentially the outlet buffer for the CE and can adversely affect the separation if it is not matched reasonably well to the running buffer. In practice, therefore, most CE–MS separations have been performed in an acidic buffer including acetic acid and ammonium acetate with a sheath liquid of water with alcohol or acetonitrile containing 0.1–1.0% acetic acid. Unfortunately, these conditions severely constrain the range of samples that can be successfully analyzed by CE–MS.

In this study, we examine suitable conditions for CE–MS of a range of small molecules. Alternative sheath liquids are compared, and various possible separation buffers are demonstrated. Both positive and negative ionization modes are tested.

2. Experimental

2.1. Materials

All reagents were of the highest purity commercially available. Buffers were freshly prepared using Milli-Q water (Millipore, Bedford, MA, USA). Details of sample and buffer composition are described in the appropriate figure legends. The capillaries were 100 cm×75 μm I.D. unmodified fused silica (Polymicro Technologies, Phoenix, AZ, USA). Samples were purchased from Sigma (St. Louis, MO, USA).

2.2. Instrumentation

All separations were performed with a Crystal 310 CE system (Thermo Capillary Electrophoresis, Franklin, MA, USA). The commercial system was used without modification and without electrical or physical interface to the mass spectrometer.

The electrospray ionization source (Analytica of Branford, Branford, CT, USA) was fitted with a CE-Pro probe (Analytica of Branford) to correctly position the capillary [13] and to provide sheath liquid. The original optics of the ion source were replaced with an Iris ion guide (Analytica of Branford) to improve sensitivity with low-mass species. The sheath flow was delivered with a Harvard Model 22 syringe pump (Harvard Apparatus, South Natick, MA, USA).

The mass spectrometer was an HP 5989A MS Engine (Hewlett-Packard, Palo Alto, CA, USA), equipped with a high-energy conversion dynode detector.

3. Results and discussion

3.1. Sheath liquid effects

The sheath liquid must be sufficiently conductive to complete the CE separation circuit and to permit ESI, but not so high in ionic strength as to create arcing and discharges in the ESI source. Furthermore, it must be low in surface tension to allow the formation of a Taylor cone while being miscible with the CE separation buffer. A variety of fluids have been used to meet these requirements, typically alcohol/water mixtures containing a conductive modifier such as acetic acid. Thus, a series of experiments were performed to optimize the sheath liquid composition for the intended CE–MS experiments.

A simple mixture of peptides (480 fmol each) was separated using a CE buffer of 0.1% acetic acid with 100% methanol as the sheath liquid (Fig. 1a). For this experiment, the CE voltage used was +30 kV and the mass spectrometer was scanned from 100 to

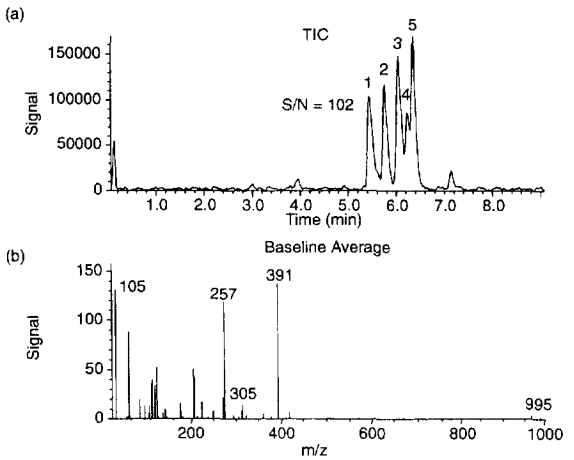

Fig. 1. (a) TIC from CE separation of peptide mixture, 480 fmol per component. (b) Mass spectrum of baseline noise, average from 3 to 4 min. Peaks: (1) Arg–Gly–Asp, (2) morphiceptin, (3) Val–Pro–Leu, (4) leucine enkephalin, (5) methionine enkephalin. CE buffer, 10 m*M* acetic acid, pH 4.6; CE potential, 30 kV; MS scan range, 100–1000 *m/z*; sheath liquid, 4 μl/min of pure methanol.

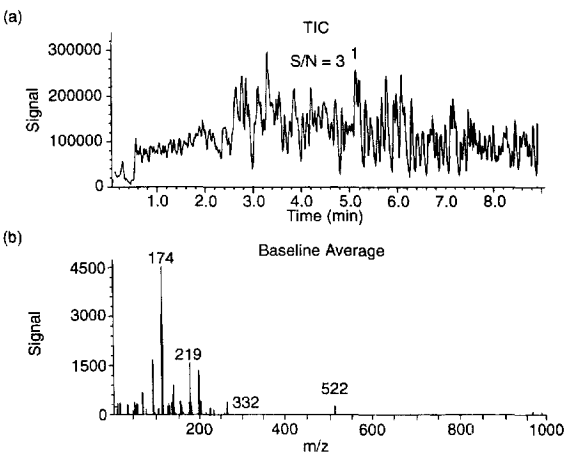

Fig. 2. (a) TIC from CE separation of peptide mixture, 480 fmol per component. (b) Mass spectrum of baseline noise, average from 3 to 4 min. Sheath liquid, 4 μl/min of methanol with 0.1% acetic acid. All other conditions same as Fig. 1.

1000 *m/z* units. Fig. 1a shows the total ion chromatogram (TIC) collected from this reproducible experiment with a signal-to-noise ratio (*S/N*) of 102 for the *m/z* 347 (Arg–Gly–Asp) peak. From the ease and reproducibility with which these data were collected, it is clear that pure methanol is sufficiently conductive to ground the CE circuit and to permit ESI, presumably because no special precautions were taken against the absorption of atmospheric water by methanol. The baseline mass spectrum, averaged between 3 and 4 min (Fig. 1b), shows a low abundance of specific chemical contaminants.

The result of adding 0.1% acetic acid to the methanol sheath, while keeping all other conditions the same, is shown in Fig. 2a. Here, the *S/N* for Arg–Gly–Asp has fallen to 3, and the peaks can no longer be easily observed in the TIC. The mass spectrum of the baseline in Fig. 2b shows a large increase in background noise. This loss of sensitivity results, therefore, from increased chemical noise due to the addition of acetic acid to the sheath rather than from loss of analyte signal. By restricting the scanned mass range to 300–1000 *m/z* units and thus excluding these low-molecular-mass species, *S/N* for Arg–Gly–Asp can be increased to almost 200 (Fig. 3a). The baseline spectrum (Fig. 3b) shows a low abundance of the same background ions, similar to that observed without acetic acid in the sheath. Using the restricted scan, it is apparent that acidifying the sheath has little or no effect on the separation itself. Although restricting the scan mass range eliminates the predominant chemical noise, this approach is unacceptable for the analysis of small molecules

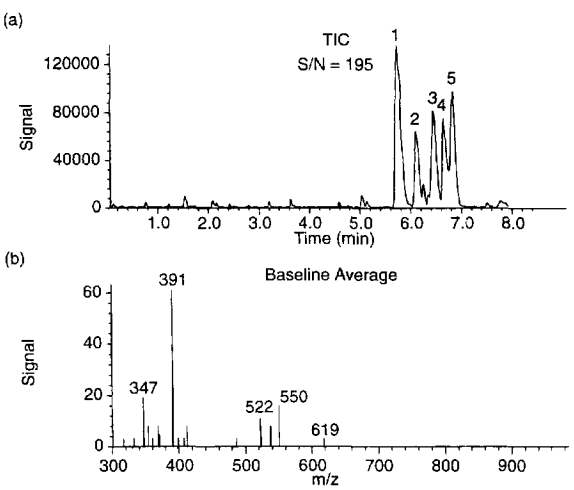

Fig. 3. (a) TIC from CE separation of peptide mixture, 480 fmol per component. (b) Mass spectrum of baseline noise, average from 3 to 4 min. Sheath liquid, 4 μl/min of methanol with 0.1% acetic acid. MS scan range, 300–1000 *m/z*. All other conditions same as Fig. 2.

whose m/z fall within this region. Upon considering these results, pure methanol was used as the sheath liquid in all subsequent experiments.

3.2. Peptide separation development

A different peptide standard was separated in a common CE–MS buffer, 10 m*M* ammonium acetate at pH 4.6 (Fig. 4). The data are presented as reconstructed ion chromatograms (RICs) in order to simplify interpretation of the results. The 350 and 524 m/z ions are the triply- and doubly-charged ions of angiotensin, respectively, and show the expected severe tailing of this basic peptide in an acidic buffer. The 380 m/z peptide (Val–Tyr–Val) co-migrates with angiotensin, while the Leu- and Met-enkephalins (556 and 574 m/z) are well-resolved. Such a separation would often be modified by small adjustments between pH 3 and 5 in order to increase resolution, even though greater changes in electrophoretic selectivity would be expected at alkaline pH. This approach, however, has not been widely used in CE–MS because of an anticipated difficulty of spraying the carbonate buffer and of the possible loss of signal from lack of protons.

Nonetheless, the same peptide mixture was separated in 10 m*M* ammonium bicarbonate at slightly alkaline pH 8.0. The result is shown in Fig. 5, again as the RICs of the particular compounds. The doubly-charged angiotensin (m/z=524) now appears as a well-resolved symmetrical peak, although the Val–Pro–Leu (m/z=380) peptide co-migrates with Leu- and Met-enkephalin. Overall, the sensitivity for the ions is about the same, except for the triply-charged angiotensin ion at m/z=350 which has completely disappeared, and doubly-charged angiotensin ion at m/z=524 gives better *S/N*. This result is not surprising as the more basic buffer used should favor the formation of a gas-phase ion with less positive charge by creating a charge state in solution with less positive charge. Relationships between solution chemistry and ESI signal have been thoroughly studied and found to correlate well [14–16]. The successful use of this buffer indicated that even higher pH could be tested. These results are shown in Fig. 6, where a buffer of pH 10.4 was used. All four peptides appear as symmetrical peaks, and there is little change in sensitivity. The expected alteration in electrophoretic selectivity is also apparent. The 380 m/z peak which co-migrated with angiotensin as the first peak at buffer pH 4.6 is now

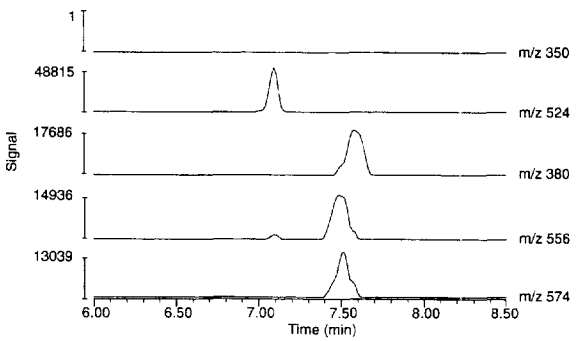

Fig. 5. RICs from CE separation of peptide mixture, 480 fmol per component. CE buffer, 10 m*M* ammonium bicarbonate, pH 8.0. All other conditions same as Fig. 4.

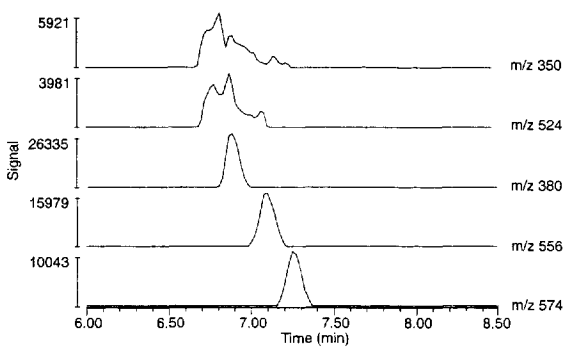

Fig. 4. RICs from CE separation of peptide mixture, 480 fmol per component. CE buffer, 10 m*M* ammonium acetate, pH 4.6; CE potential, 30 kV; sheath liquid, 4 μl/min pure methanol.

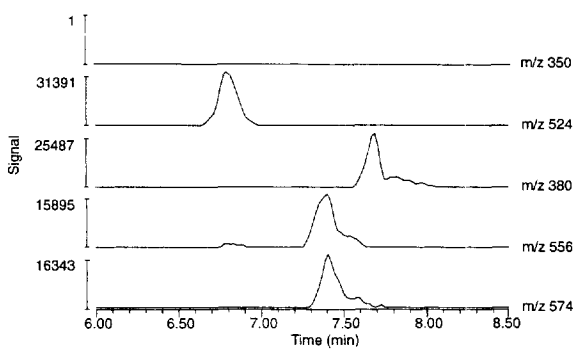

Fig. 6. RICs from CE separation of peptide mixture, 480 fmol per component. CE buffer, 10 m*M* ammonium bicarbonate, pH 10.4. All other conditions same as Fig. 4.

well-resolved and has the longest migration time of all the ions. On the other hand, the enkephalins which were resolved at low-pH buffer now co-migrate with the higher pH buffer.

3.3. Separation of basic drugs

The successful use of high-pH buffers for peptides suggests an approach for the CE–MS analysis of basic drugs as well. Such compounds give the best separations near the pK values, slightly above pH 9. The ionic strength of the buffer must also be relatively high, both to eliminate wall interactions and to reduce electroosmotic flow. A mixture of over-the-counter cough syrup medications was separated in 100 mM ammonium bicarbonate at pH 8.0 and 9.2. (Fig. 7a and Fig. 7b). As expected, the mixture of basic drugs is considerably better resolved with the higher pH buffer. The behavior of guaifenesin and acetaminophen is particularly interesting. Guaifenesin is neutral in solution and migrates at the position of the neutral marker. It gives a substantial positive ion response in electrospray. Acetaminophen is negatively charged, reflecting deprotonation of the phenolic hydroxyl, yet it also yields a positive ESI ion. In both cases, the observed ion corresponds to M+1 with no evidence of rearrangement or fragmentation. This useful phenomenon requires further investigation and may be partially explained by considering the results from Van Berkel [17] who has shown that the pH, and thus $[H^+]$, in the bulk solution may be vastly different from the pH of the significantly evaporated ESI droplet from whence the gas-phase ion is extracted.

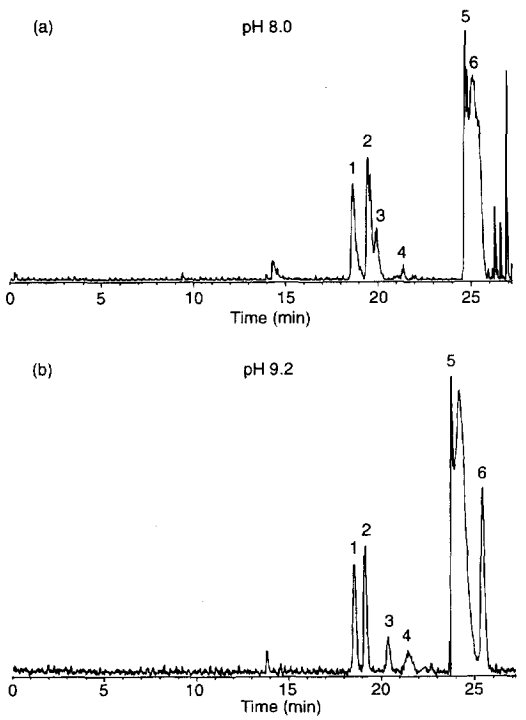

Fig. 7. TIC from separation of cough syrup components: (a) pH 8 and (b) pH 9.2. Peaks: (1) pseudoephedrine, (2) dextromethorphan, (3) phenylpropanolamine, (4) pyrilamine, (5) guaifenesin, (6) acetaminophen. CE Buffer, 100 mM ammonium carbonate; CE voltage, 17 kV.

3.4. Negative ionization

The above successful use of high-pH separation electrolytes suggests an approach to analyzing anionic analytes migrating counter to the electroosmotic flow. In order to use the ESI source in the negative ion mode, oxygen was used as a sheath gas to suppress electrical arcing by corona discharge. A series of alkyl sulfonates was selected as a model of singly-charged anions and was separated (Fig. 8). The overlaid RICs show symmetrical peaks for each of the analytes. The order is that expected for counter EOF migration of anions, and there is no evidence of aggregation, a problem frequently en-

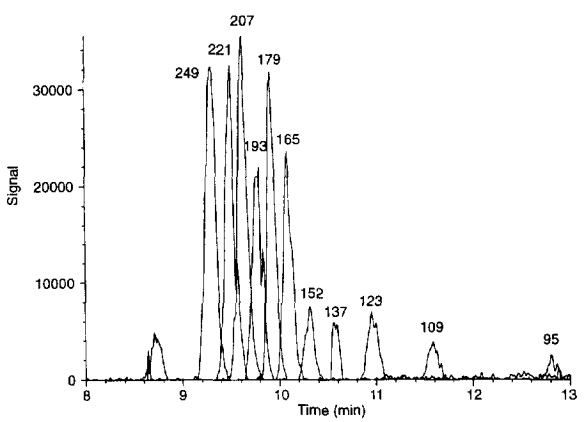

Fig. 8. RICs from separation of alkyl sulfonates. All other conditions same as Fig. 7b; m/z ions are as indicated.

countered when separating these species by LC. Although all the analytes were present at the same molar concentration, response is better for the longer chain, more hydrophobic alkyl-sulfonates. This again may be a the result of solution chemistry effects. According to the ion evaporation model advanced by Iribarne and Thomson [18], the ion in the gas phase is extracted from the ESI droplet surface by the large electric field generated in the atmospheric region of the ion source. Assuming this hypothesis to be correct, it is reasonable to expect stronger ESI signals from compounds which are more surface active. Increasing the length of the alkyl chains on these sulfonates increases their surface activity accordingly, and thus may increase their ESI–MS signal as well.

A mixture of food dyes was next used as a model for small analytes with multiple negative charges. Fig. 9a shows the TIC from a separation of a mixture of four such dyes, while Fig. 9b shows the averaged mass spectra corresponding to each of the four peaks. The first peak is FD&C Blue 1. It has one quaternary ammonium and three sulfonyl groups. The major ion observed at 373 m/z corresponds to $M-2H^+$ and thus has an overall charge of -2. A smaller singly-charged negative ion signal is given at $m/z=748$, the result of $M-H^+$. FD&C Red 3, the next peak from the TIC, shows a predominant $M-1$, although in solution it has two negative charges from a carboxyl and a fully ionized phenolic hydroxyl. The doubly-charged species is the major product of FD&C Red 40, the third peak in the separation, and has two sulfonyl groups. Finally, FD&C Yellow 5 gives roughly equal yields of the singly- and doubly-charged ions for its two sulfonyl groups and one carboxyl group. In all four cases, the observed partially charged ions correspond to protonation of the acidic groups, with no evidence of sodiated or ammoniated species. The yield of alternative charge states in these mass spectra may depend on the chemical structure, particularly the three-dimensional arrangement of the acidic groups and thus their availability for protonation. Also, as previously discussed, the pH and thus chemical equilibria of the evaporated ESI droplet may differ substantially from that of the bulk solution.

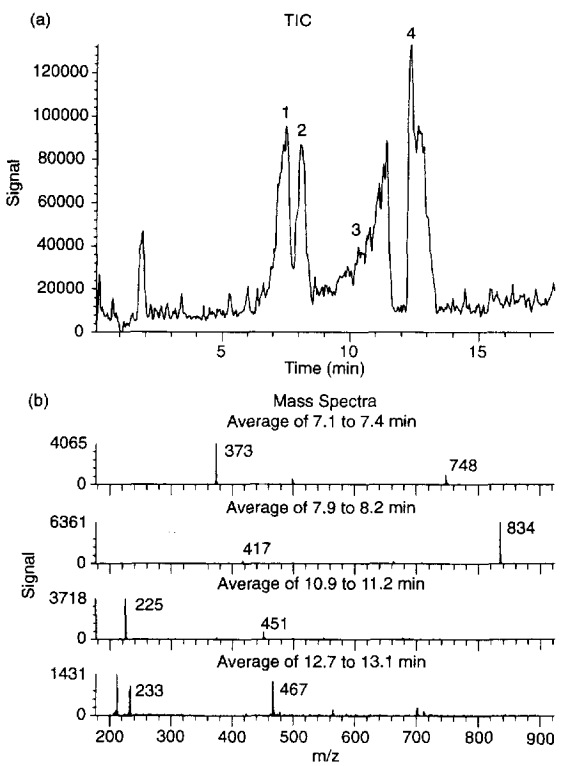

Fig. 9. (a) TIC and (b) mass spectra for separation of food dyes. All other conditions same as Fig. 7b.

4. Conclusions

These experiments show an approach to optimizing chemical parameters for CE–MS with ESI. It has proven straightforward to effect this technique using commercially available hardware without modification. Sensitivity is more than adequate for a variety of applications. A wide range of electrophoresis buffers were compatible with ESI, providing many options for optimizing the separation. Both positive and negative ESI were successfully demonstrated.

The major observed sensitivity limitation was chemical noise derived from the addition of acetic acid to the sheath liquid. Omitting both acid and water from the sheath gave better signal-to-noise response. A pure methanol sheath liquid was used for all experiments, and no interference with the separation was observed. Certainly, mismatches between the separation and sheath liquids are expected to have an effect on the observed separation. How-

ever, there is no obvious need to use the same buffer for both purposes. Most important, during methods development experiments, it is not necessary to continuously adjust the sheath to accommodate the tested electrolytes.

A wide range of buffers was compatible with CE–MS. Common practice has focused on low-ionic strength, low-pH electrolytes to ensure a good yield of analyte ions, even at the expense of separation quality. We have found good sensitivity for analytes at pH values where they were at best partially protonated in solution. In addition, high-ionic strength separation buffers did not markedly interfere with ESI, even with currents as high as 20–30 μA.

These observations suggest many options for improving CE–MS analyses by manipulating the pH and ionic strength of the separation buffer, thus altering the solution chemistry. This approach can extend the use of this technique to a wide range of small molecule analytical problems.

Acknowledgments

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