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General considerations for optimizing a capillary electrophoresis–electrospray ionization time-of-flight mass spectrometry system

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Abstract

Modern analytical instrumentation must be able to perform rapid, reliable, and sensitive analysis. The on-line combination of analytical techniques such as capillary electrophoresis (CE), electrospray ionization (ESI) and mass spectrometry (MS) can provide solutions to numerous problems related to complex mixtures of organic/inorganic, or biological materials. Optimum performance of complex instrumentation such as this can be achieved only if each individual component operates with maximum proficiency, and is in full harmony/compatibility with the other components. The present paper reports on the evaluation and optimization of a CE-ESI time-of-flight mass spectrometry (TOF-MS) system. The main features of the instrument are speed and sensitivity. Low amol (3–11) detection limits have been achieved with continuous infusion experiments, and the acquisition rate can be as high as 10 000 spectra s^{-1}. For CE–TOF-MS, minimum detection was in the very-low fmol (1–10) range. The major contributing factors to high quality analysis characteristic to each separate technique are considered, relevant examples are discussed, and fast, and sensitive analysis is demonstrated.

Keywords: Capillary electrophoresis–mass spectrometry; Optimization; Instrumentation; Paraquat; Gramicidin S; Enkephalins

1. Introduction

The interfacing of highly efficient capillary electrophoresis (CE) separation methods to sensitive, and information-producing detection systems such as mass spectrometry (MS) results in an extremely valuable tool, rivaled only by gas chromatography (GC)–MS and liquid chromatography (LC)–MS. In terms of sensitivity, the sample concentration detection limits for CE–MS are slightly better than for CE–UV (~10^{-5}–10^{-6} *M*) [1], however, preconcentration techniques such as membrane preconcentration, capillary isotachophoresis (cITP)–CE, LC–CE, sample stacking and field amplified injection can bring 100–1000-times improvements in these limits. Laser-induced fluorescence (LIF) for CE has provided detection at 10^{-8}–10^{-10} *M* concentration, and at the yoctomol level [2], however, the necessity for derivatization of the majority of compounds limits its attractiveness.

In addition to interfacing CE to commonly used mass spectrometers such as quadrupole or ion trap instruments, the last several years have seen studies

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involving ion mobility spectrometry, plasma desorption and matrix-assisted laser desorption ionization (MALDI) MS, quadrupole ion trap Fourier transform ion cyclotron resonance (FT-ICR)-MS, double focusing MS, and tandem MS [3–5]. Besides sensitivity, another important characteristic of CE–MS is speed. Fast separations which yield peak widths of several ms are frequently reported in CE [6,7]. The accurate monitoring of these peaks, along with reliable quantitation, can be accomplished only if the mass spectrometer is capable of producing a sufficient number of data points across the peak width, with each data point representing a full mass spectrum. The majority of mass spectrometers cannot produce mass spectra at this rate. Scanning or ion trapping instruments need 0.1 to 2 s to produce a full mass spectrum.

The unquestionable advantage of TOF-MS [8,9] is that an extremely short time (10–200 μs, depending on the instrumental design and pulsing rate) is necessary to produce a full mass spectrum. In order to obtain a clear picture of the required scan speed for monitoring a chromatographic peak, it can be assumed that a minimum of ten data points across the peak will be sufficient for the accurate characterization of the peak profile. If the peak width is either 0.1 or 0.01 s in width, then a sampling frequency of 100 or 1000 Hz, respectively, will be required, allowing only 10 or 1 ms for producing a mass spectrum. These short acquisition times are beyond the capabilities of all current mass spectrometers, except for the TOF. Mass spectral acquisition time is important in comparing the sensitivities of various mass spectrometers. For example, in an ESI-FT-ICR experiment, 5 amol of analyte (ubiquitin) produced a recognizable mass spectrum in 95 s (100 μs gated trapping, followed by 95 s for base pressure restoration [10]). For comparison, a quadrupole instrument was able to detect only 35 zmol of consumed neurotensin, however 8 s were necessary to collect the spectrum [11].

The ionization sources used in CE–MS were initially developed for LC–MS. The choice of interface is mainly determined by the nature of the analytes, the mobile phase, and the mobile phase flow-rate. The most commonly used interface for CE–MS is ESI, which has been applied to all CE modes of operation. The ESI technique initially proposed by Dole et al. [12,13] has been further developed by Iribarne and Thomson [14], and Fenn and co-workers [15–17] and has become popular due to some of its inherent advantages: simplicity (brings ions directly from the liquid phase into the gas phase), high ionization efficiency (50–100%), and ability to produce multiply-charged ions (high mass ions, M_r $110 \cdot 10^6$ [18], so that m/z ratios fall within the operating range of quadrupole MS instruments). In cases where high concentration buffer systems, especially nonvolatile buffers, significantly diminish the ESI analyte signal, and high concentration surfactants typical in micellar electrokinetic chromatography (MEKC) result in analyte signal suppression, the atmospheric pressure chemical ionization (APCI) source has proven to be more suitable for CE–MS [19,20]. Continuous flow fast atom bombardment (CF-FAB) sources are less common than ESI; they are less sensitive, the background noise is higher, and they have lower applicability to high-molecular-mass compounds [21]. Inductively coupled plasma (ICP) sources are used for trace metal analysis [5].

CE–ESI-MS was first reported with quadrupole instruments in 1987 [22–25], while CE–TOF-MS has been investigated only since 1994 [26–35]. We have recently reported on the design and operation of an ESI-TOF-MS system [36,37]. This paper reports the results of a series of experiments performed to improve the overall performance of the system, particularly with respect to high sensitivity detection for CE.

2. Experimental

2.1. Reagents

Standard samples were prepared in HPLC-grade solvents. Acetonitrile, methanol and water were purchased from Mallinckrodt (Chesterfield, MO, USA). Glacial acetic acid was obtained from EM Science (Gibbstown, NJ, USA). Paraquat dichloride (methyl viologen dichloride hydrate, 98%) and ammonium acetate (99.999%) were purchased from Aldrich (Milwaukee, WI, USA). Dibasic anhydrous sodium phosphate (Na_2HPO_4) was obtained from Mallinkrodt, and monobasic potassium phosphate (KH_2PO_4) was purchased from EM Science (Cherry

Hill, NJ, USA). Peptides were purchased from Sigma (St. Louis, MO, USA). Samples were prepared by dissolving the appropriate amount of analyte in a mixture of CH_3OH–water, or CH_3OH–water–CH_3COOH.

2.2. Instrumentation

2.2.1. Capillary electrophoresis

CE was performed on a Crystal CE 300 system (ATI, Madison, WI, USA) using an Applied Biosystems (Foster City, CA, USA) Model 785A UV detector. For fast separations which required the use of short capillaries, a small laboratory-built CE system was used. The CE column and high voltage electrode were introduced into a 5-ml CE inlet vial through a silicone septum and special adaptor, which also allowed for application of the necessary head pressure for sample introduction or capillary rinsing. The head pressure was applied from a nitrogen gas cylinder through a series of two regulators and two valves. A calibrated meter (Model DP25-S, Omega, Stamford, CT, USA), and a PX137-001 DV pressure sensor (Omega) operating under 1 p.s.i. (1 p.s.i.= 6894.76 Pa), provided a good indication of the pressure level during sample introduction. Uncoated fused-silica capillaries (50 μm I.D.×190 μm O.D.) from Polymicro Technologies (Phoenix, AZ, USA) were conditioned prior to analysis by rinsing for 10–15 min with sodium hydroxide (1 *M*), followed by HPLC water for 10–15 min. Continuous infusion of the analyte solutions was achieved with a Harvard 22 syringe pump (South Natick, MA, USA). The standard solutions, CE buffers, and sheath liquid were degassed by sonication prior to use.

2.2.2. ESI-TOF-MS

Detailed descriptions of the TOF instrument and the ESI source are given in previous papers [36,37]. Ions produced in the ESI source are sampled through a nozzle/skimmer arrangement. A heated countercurrent of nitrogen gas fed between the interface plate and the sampling nozzle ensures the drying of the electrosprayed droplets. Two sets of radio frequency (RF)-only quadrupoles ensure efficient transmission of the ion beam inside the differentially pumped vacuum stages. The ions were determined by operating the TOF-MS system in the linear mode after orthogonal extraction into the flight tube from a Wiley–McLaren pulsing region. A schematic diagram of the ESI-TOF-MS system is given in Fig. 1.

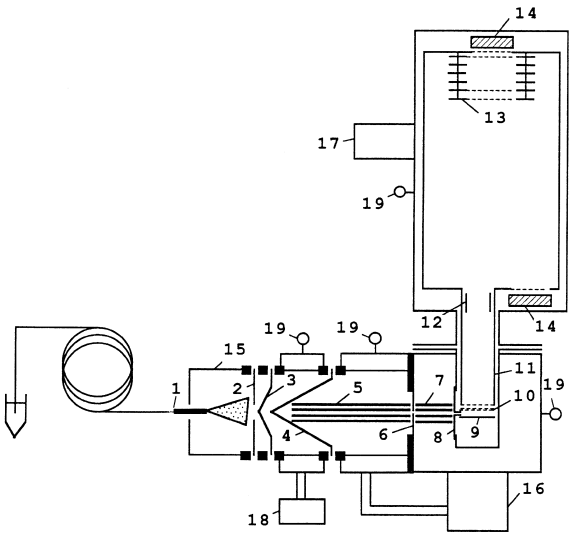

Fig. 1. Schematic diagram of the ESI-TOF-MS system. (1) Electrospray needle, (2) interface plate, (3) nozzle, (4) skimmer, (5, 7) RF-only quadrupoles, (6) interquad lens, (8) slit/lens, (9) pulser, (10) grids, (11) flight tube, (12) deflection plates, (13) reflectron, (14) electron multiplier, (15) ion source, (16, 17) turbo pumps, (18) rotary pump, (19) vacuum gauge.

3. Results and discussion

Sensitive analysis in CE–ESI-MS can be achieved only if each contributing component of the system is properly optimized to give its best performance. The injection, separation, ionization, ion sampling, ion transmission, detection and data processing, are individual steps which bring separate contributions to the quality of the final result.

3.1. Capillary electrophoresis

The efficiency of a separation indicates the ability of the method and instrumentation to separate individual components into very narrow bands/zones. Band broadening in CE results in wider and lower intensity peaks, and loss of resolution and sensitivity. Band broadening can be produced by different components of the CE system; it can be injection

related, detection related, or separation related (diffusion, convection and electrodispersion). Analytes in the original sample solution should be prepared in a much lower concentration than the CE buffer (at least 100-times) to avoid broadening due to anti-stacking effects.

Typically CE is performed using 50 μm I.D. capillary columns, which present a good compromise between separation efficiency and loading capacity. A common mistake is to overload the CE column with sample. The injected sample plug length in CE should be less than 0.2% of the column length in order to prevent significant peak broadening. This translates into injection volumes of only several nl, and explains why CE has poor concentration limits of detection but excellent mass limits of detection compared to LC [38]. In Fig. 2 we compare the injection of approximately a 0.8 mm plug (1.5 nl) to a 5 mm plug (9 nl) of 40 μ*M* solution of leucine enkephalin. For a six-times larger volume, the signal intensity increased only two times, while the efficiency of the peak decreased from 105 000 plates to 6200 plates. In the case of a real separation, this result would seriously compromise the resolution of closely eluting peaks, and would significantly increase the detection limits. Large volumes of a dilute sample can be injected in CE only if the sample buffer solution is properly chosen relative to the CE buffer to provide adequate compression of the analyte zones.

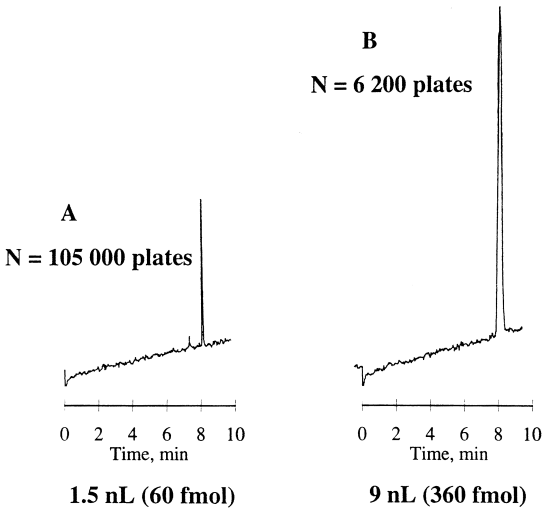

Fig. 2. CE–UV electropherograms illustrating the effect of injection volume on peak intensity and width for leucine enkephalin. Conditions: leucine enkephalin (40 μ*M*) in CH_3OH–water–CH_3COOH (50:50:0.03, v/v); 90 cm×50 μm I.D. uncoated fused-silica capillary, CH_3CN–water–CH_3COOH (50:50:0.3, v/v), 20 kV, 0.2 μA, 210 nm at 75 cm; (A) 15 mbar×0.1 min injection; (B) 30 mbar×0.3 min injection.

If we consider what happens in the CE column during a separation, we must take into account numerous factors which contribute to the final peak shapes and to the resulting sensitivity. A CE separation occurs in the presence of a buffering system. The buffer allows precise pH control of the CE carrier and provides the ionic strength necessary for electrical continuity. At high buffer concentrations, sharp peaks are produced as a result of stacking, the interactions between the analyte and the capillary wall are reduced, and the linear dynamic range and sample loading capacity are increased [38]. The disadvantages of using high concentration buffers are that larger currents flow through the capillary, which generates Joule heating, temperature gradients across the column, lower viscosity eluents, and overall, results in changes in the electroosmotic flow (EOF), electrophoretic mobilities and diffusion coefficients of the ionic species, which ultimately deteriorates the quality of the separation. At low buffer concentrations, faster separations are possible and larger diameter capillaries can be used due to reduced Joule heating. In addition to the buffer, various additives can be introduced into the eluent in order to modify the capillary inner surface and the EOF, and to reduce wall interactions.

Detector related factors which may induce band broadening are the detector time constant and the length of the detector window. In this case, the detector is the ESI-TOF-MS system for which special requirements apply.

3.2. Electrospray interface

Liquid sheath and microspray sources have been used in this study. The construction and optimization of these interfaces have been described previously [37]. From the point of view of a separation scientist, the main requirements of the interface are high analyte transfer efficiency and preservation of the separation efficiency and resolution [39]. Other aspects are desired as well, such as free choice of

column dimensions, eluent composition, flow-rate and ionization method (±), and close control of all chemical changes within the interface.

Ion transport in the ion source occurs through three major mechanisms: convective, diffusive and electrostatic [40]. In an intense ion source, which produces a concentrated ion beam, once the ions are formed, they rapidly start to spread due to mutual coulombic repulsion, resulting in reduced ion current; ion beam divergence occurs mainly through radial diffusion, ion mobility in the externally applied fields, and space charge repulsion.

The focusing at atmospheric pressure of a divergent ion beam is a difficult task. Applying a voltage to the ion source interface plate (600 V) was able to increase the signal intensity by 30–50%. However, the major concerns when ESI is used as an ionization source for CE are related to the CE system itself. Three important factors must be considered: the separation pH, the nature and concentration of the buffer, and the presence or absence of EOF. The pH of the eluent determines the net charge on a given molecule. At low pH, the formation of positive ions is favored. At high pH, negative ions are produced. In untreated fused-silica capillaries, the EOF is strongly dependent on the pH and on the buffer concentration. At low pH (pH 2–3), the EOF is very small (0–30 nl min^{-1}), while at high pH (pH 8–9), it can reach 200–300 nl min^{-1} for a 50 μm I.D. capillary [38]. High buffer concentrations will reduce the EOF. The present capillary column technology allows for efficient control of EOF. Capillary inner walls can be activated or coated to produce constant EOF over a wide pH range, no EOF, or variable EOF. Specific coatings and/or buffer additives can also minimize the adsorption of analytes on the wall. The nature of the capillary selected for use is determined by the specific application of interest. Low or no EOF usually results in increased separation efficiency, which is desirable in CE related techniques such as isotachophoresis or isoelectric focusing. On the other hand, large EOF results in much faster separations and allows the separation of positive and negative ions in one run.

The above-mentioned considerations determine the choice of electrospray source. It is well known that there is a fundamental incompatibility between optimal CE and optimal ESI operation. The nonvolatile, high concentration buffers from the CE column can result in complete signal suppression in the ESI source. The liquid sheath has two roles: to apply the necessary electrical potential to the capillary column terminus for CE electrical continuity and for simultaneously creating the electrospray, and to dilute the high concentration aqueous buffer with an organic solvent to make it electrosprayable. With a liquid sheath source, nonvolatile buffers such as citrate, phosphate, or even sodium dodecyl sulfate have been electrosprayed. Analytes separated as negative ions in the CE capillary can be electrosprayed as positive ions if the liquid sheath is able to reverse the charge on the molecule.

If the liquid sheath source is used, the liquid sheath flow-rate and the alignment of the CE capillary in the spray needle can seriously compromise the original separation efficiency and resolution for fast and narrow eluting peaks. The liquid sheath is known to attenuate the signal intensity by itself several times [37,41] compared to a microspray source, especially when used at high flow-rates (5–10 μl min^{-1}).

The microelectrospray is a more efficient ionization source than the liquid sheath and can considerably improve the sensitivity of the ESI technique. Its application in CE–MS interfacing is highly desired, however, only volatile and low concentration buffer systems (ammonium acetate or formate) can be electrosprayed. It is necessary to have a positive EOF in the CE capillary in order to sustain the spray. To increase the spray stability, a small pressure on the CE inlet buffer is often applied. In our work, we always applied a 10 mbar pressure on the CE inlet vial, even when the liquid sheath source was used, in order to prevent penetration of the liquid sheath into the CE capillary. Hydrostatic pressure on the capillary inlet may induce laminar flows which may in turn diminish the separation efficiency. However, in our case, the migration times of the analytes usually decreased by 7–10%, and in one case we actually experienced increased separation efficiency [37]. When the microelectrospray source is used, only positive or negative ions can be determined, since there is no liquid sheath to change the charge on the ions. The main advantages of the microspray source compared to the liquid sheath are improved detection limits, and preservation of sepa-

ration efficiency and resolution. Commonly encountered drawbacks are frequent plugging of the narrow I.D. (2–5 μm) spray tips and reduced lifetime of the commonly used gold coatings.

3.3. Time-of-flight mass spectrometry

Ions which are formed at atmospheric pressure and are drawn inside the mass spectrometer must be efficiently transported with minimal loss until they reach the detector. The purpose of this research was to design and build a sensitive ESI-TOF-MS system for fast CE separations (<5 min) which can achieve detection limits in the low amol range (Fig. 1). A series of optimization studies were performed using the TOF system with the main purpose of producing maximum intensity signals and improved detection. These included evaluation of the following parameters: optimum spacing between the nozzle and the skimmer, optimum operational parameters for the RF-only quadrupoles, and optimum orifice sizes in the ion optics elements which produce ideal pressures for maximum ion focusing and transmission through the quadrupoles.

With our first configuration [0.25 mm orifice in the sampling nozzle (Nz), 0.8 mm orifice in the skimmer (Sk), 0.75 mm orifice in the interquad lens (IQL), 1 mm orifice in the slit/lens (Sl) between the third and fourth vacuum stages, 1.5–2 mm spacing between the nozzle and skimmer and f=1.96 MHz/$V_{0\text{-peak}}$=500–600 V on the two sets of RF-only quadrupoles], the following results were obtained [36]: (a) the resulting pressures in the four vacuum stages were 3 Torr in the first stage, $1.5\cdot10^{-1}$ Torr in the second stage, $7\cdot10^{-6}$ Torr in the third stage, and $(3–4)\cdot10^{-7}$ Torr in the fourth stage (1 Torr=133.322 Pa); (b) approximately 40–45% of the ion beam was focused inside the 0.75 mm orifice IQL, resulting in a very good detection level for gramicidin S (30 amol) from a 0.1 μM solution; and (c) poor signals were generated from low m/z ions (e.g., Paraquat, m/z =93).

Dodonov et al. [42] have shown that the maximum ratio of ion current per neutral gas flow through the skimmer orifice was achieved for a Nz/Sk spacing of −2.5M (M=distance from Nz orifice to Mach disc), and Douglas and French [43] have demonstrated that maximum collisional focusing and ion transmission of the RF-only quadrupoles is achieved at $(6–8)\cdot10^{-3}$ Torr. By increasing the pumping capacity in the first vacuum stage (two E2M18 Edwards rotary pumps were used instead of one), by changing the orifice sizes in the ion optics elements (0.25 mm Nz, 0.5 mm Sk, 3.2 mm IQL, and 1 mm Sl), and by increasing the spacing between the Nz and Sk to

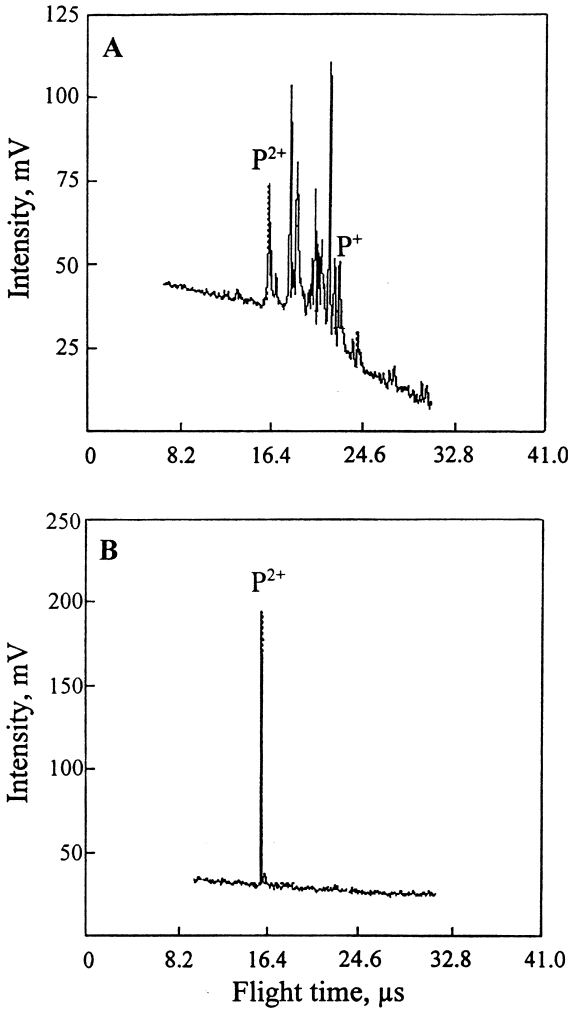

Fig. 3. Effect of MS configuration on TOF mass spectrum of paraquat. Conditions: Paraquat (50 μM) in CH_3OH–water–CH_3COOH (70:30:0.1, v/v); continuous infusion (0.3 μl min^{-1}); μESI (2150 V), 40°C; MS data acquisition at 5000 Hz pulsing rate, 5000 spectra averaged, 1 spectrum s^{-1}; (A) Nz/Sk (0.6M), electron multiplier (−2500 V), P_{quad} ($1.2\cdot10^{-1}$ Torr); (B) Nz/Sk (2.1M), electron multiplier (−2000 V), P_{quad} ($3\cdot10^{-2}$ Torr). The flight time range corresponds to 0–603 m/z.

about 6–7 mm (2*M*), we were able to achieve a more favorable pressure distribution for ion transmission in the mass spectrometer [2 Torr in the first stage, $(6–7)\cdot10^{-3}$ Torr in the second stage, $1.5\cdot10^{-5}$ Torr in the third stage and $(4–5)\cdot10^{-7}$ Torr in the fourth stage], and improved ion transmission and sensitivity. An intermediate set-up, similar to the second configuration, but with the original 0.75 mm orifice IQL, was tested as well. This allowed the observation that the Nz/Sk spacing and the optimized pressure in the first quadrupole were responsible for the improved transmission. The larger IQL (3.2 mm) did not significantly affect the throughput of ions, but rather helped in lowering the pressure in the second vacuum stage.

Ion sampling and transmission with the intermediate and second configurations improved mainly the performance for low *m*/*z* ions. The paraquat ion which was essentially lost initially in a series of low intensity ion clusters, began to display a clear spectrum with a strong intensity peak at 93 *m*/*z*, corresponding to the doubly charged ion; the corresponding singly charged ion (186 *m*/*z*) had a very low intensity, or was not even visible [Fig. 3: (A) collected with the first MS configuration and (B) collected with the intermediate MS configuration]. The effect of pressure in the second vacuum stage on the transmission of the first RF-only quadrupole was studied by gradually closing a spindle valve between the third and second pumping stages, which allowed for an increase in pressure in the second stage while keeping the physical configuration of the mass spectrometer unchanged (Fig. 4, data collected with the intermediate MS configuration). Increasing the pressure in the second vacuum stage from $3\cdot10^{-2}$ Torr to $1.2\cdot10^{-1}$ Torr resulted in approximately 50% loss in the paraquat ion intensity. With the second MS configuration, the behavior was similar. Usually, by changing the second vacuum stage pressure from the 10^{-3} Torr to 10^{-2} Torr range, the signal dropped by 30–50%. The loss in intensity was more pronounced for low *m*/*z* ions.

A major contribution to the efficient transport of ions through the quadrupoles was given by the elements which controlled the ion kinetic energy [Sk, IQL and quadrupole rod offset (OFF) voltages]. For optimal collisional focusing in an RF-only quadrupole, the ion injection energy must be low [43]. In

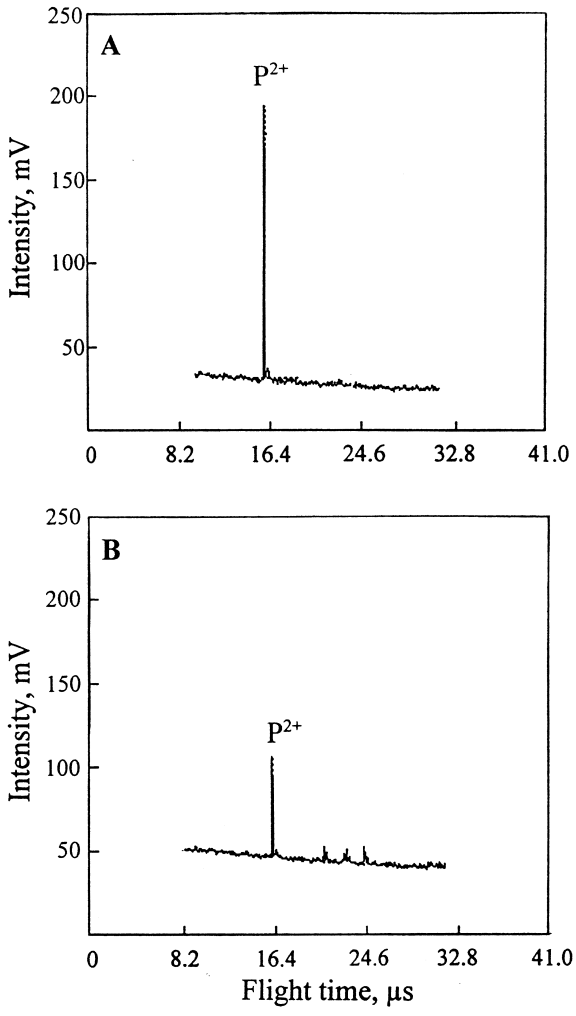

Fig. 4. TOF mass spectra illustrating the effect of pressure in the second vacuum stage on transmission of paraquat ion. Conditions: Paraquat (50 μ*M*) in CH_3OH–water–CH_3COOH (70:30:0.1, v/v); continuous infusion (0.3 μl min^{-1}); μESI (2150 V), 40°C; MS data acquisition at 5000 Hz pulsing rate, 5000 spectra averaged, 1 spectrum s^{-1}; (A) $3\cdot10^{-2}$ Torr; (B) $1.2\cdot10^{-1}$ Torr.

Fig. 5 (data collected with the first MS configuration) it can be observed that maximum focusing and transmission of the doubly charged gramicidin S ion (*m*/*z* 571) can be achieved only for a relatively narrow range of voltage settings on the critical elements. Similar results have been obtained for the paraquat ion, however the optimal voltage settings were different than those for gramicidin S. The obvious outcome was a significant mass discrimina-

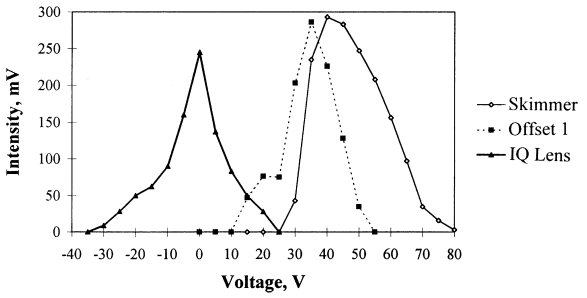

Fig. 5. Plot illustrating the effect of voltage settings on gramicidin S signal intensity. Conditions: gramicidin S (10 μ*M*) in CH_3OH–water–CH_3COOH (70:30:0.1, v/v); continuous infusion (0.3 μl min^{-1}); μESI (2100 V), 30°C; MS data acquisition at 5000 Hz pulsing rate, 5000 spectra averaged, 1 spectrum s^{-1}.

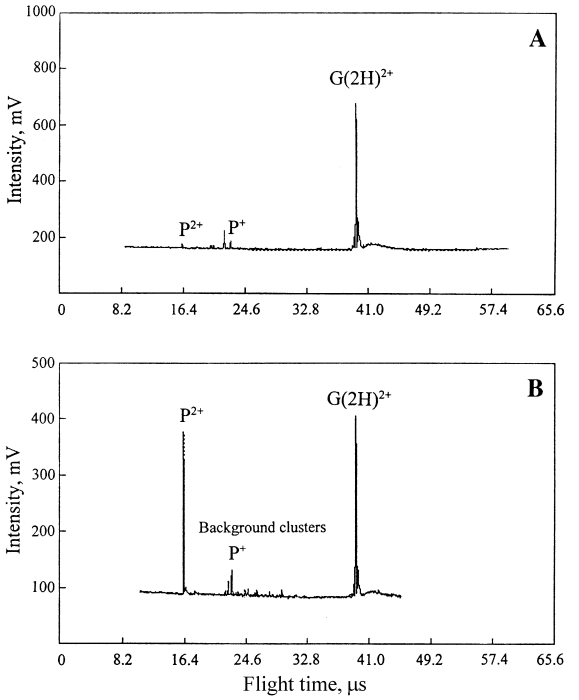

Fig. 6. TOF mass spectra illustrating the effect of mass spectrometer tuning for high and low *m*/*z* ratio ions. Conditions: Paraquat (50 μ*M*), and gramicidin S (10 μ*M*) in CH_3OH–water–CH_3COOH (70:30:0.1, v/v); continuous infusion (0.3 μl min^{-1}); μESI (2100 V), 40°C; MS data acquisition at 5000 Hz pulsing rate, 5000 spectra averaged, 1 spectrum s^{-1}; (A) mass spectrometer tuned for gramicidin S (*m*/*z* 571); (B) mass spectrometer tuned for Paraquat (*m*/*z* 93). The flight time range corresponds to 0–1545 *m*/*z*.

tion induced by these ion optic elements. If the voltage settings were optimized for high *m*/*z* ratio ions (e.g., gramicidin S, *m*/*z* 571), the signals for low *m*/*z* ratio ions (e.g., paraquat, *m*/*z* 93) were almost completely lost. If the voltages were optimized for low *m*/*z* ions, then the signal for the high *m*/*z* gramicidin S ion decreased by only 20–30% from its maximum value (Fig. 6, data collected with the second MS configuration). Consequently, simultaneous analysis of mixtures of low and high *m*/*z* ratio ions was subsequently performed at voltages optimized for low *m*/*z* ratio ions. Under optimized conditions, the paraquat ion transmission still remained several times lower than that of gramicidin S.

When performing the experiments which involved pressure changes in the quadrupole regions, the IQL and rod offset voltages had to be adjusted for each given pressure. The offset voltage on the second set of quadrupoles had to be adjusted only for the second MS configuration, when the pressure change in the second vacuum stage induced a significant pressure change in the third vacuum stage through the large, 3.2 mm orifice IQL.

These optimization tests resulted in a detection level of 3 amol of gramicidin S from a 0.1 μ*M* solution [36]. The data were collected at 10 000 Hz pulsing frequency; by averaging 100 spectra, it took only 10 ms acquisition time to produce the mass

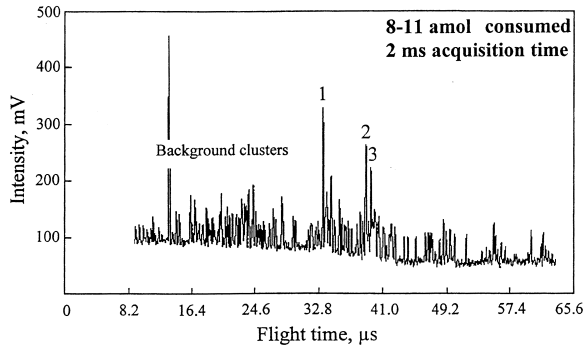

Fig. 7. TOF mass spectrum illustrating the detection level for enkephalins. Conditions: enkephalins (1.3–1.9 μ*M*) in CH_3OH–water–CH_3COOH (50:50:0.1, v/v); continuous infusion (0.2 μl min^{-1}); μESI (1700 V), 70°C; MS data acquisition at 10 000 Hz pulsing rate, 20 spectra averaged. Peak identifications: (1) des-Tyr[1],[D-Ala[2], D-Leu[5]]-enkephalin, (2) leucine enkephalin, (3) methionine enkephalin.

spectrum. From a more concentrated solution (1–2 μ*M* in enkephalins) and by averaging only 20 spectra, it was possible to collect a full mass spectrum in only 2 ms acquisition time (Fig. 7, data collected with the second MS configuration). The amount consumed was 8–11 amol. This record acquisition time at these extremely low detection levels has not been achieved by any other mass spectrometer to date.

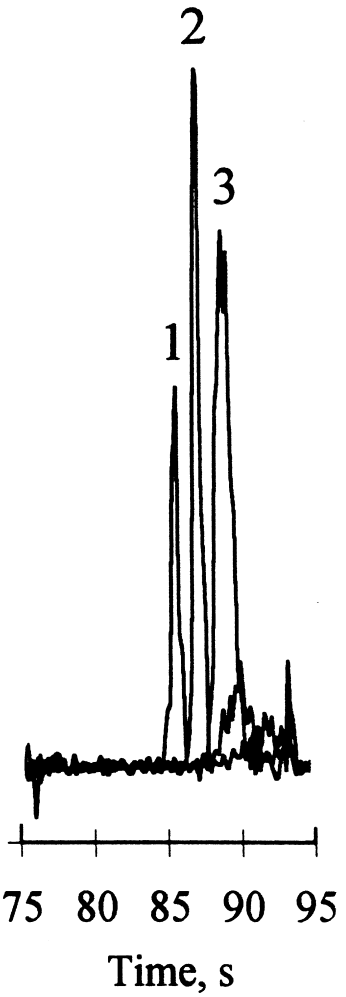

Fig. 8. Fast CE–ESI-TOF-MS electropherogram of enkephalins. Conditions: 26 cm×25 μm I.D. uncoated fused-silica, CH_3CN–water–CH_3COOH (50:50:0.3, v/v), 15 kV, 10 mbar, 0.3 μA, 20 mbar×0.06 min injection; μESI (1500 V), 80°C; MS data acquisition at 10 000 Hz pulsing rate, 6 data points s^{-1}. Peak identifications: (1) des-Tyr[1],[D-Ala[2], D-Leu[5]]-enkephalin, (2) leucine enkephalin, (3) methionine enkephalin.

3.4. CE–ESI-TOF-MS

Considering all of the previously discussed factors, the in-laboratory built CE system was interfaced to the TOF-MS system using a microelectrospray source [37]. The ESI voltage was applied through a metal union placed between the CE capillary and the ESI tip. Fast separation of three enkephalins (des-Tyr[1],[D-Ala[2], D-Leu[5]]-enkephalin acetate, leucine enkephalin acetate, and methionine enkephalin acetate) within a time window of 6 s was achieved on short capillaries (26 cm×25 μm I.D.) using acidified mixtures of CH_3CN–water as eluent (Fig. 8). Data were collected at 6 Hz sampling frequency (spectra s^{-1}). The electropherograms were rebuilt by integrating each mass spectrum within a narrow mass range corresponding to the protonated molecular ions (407, 556 and 574 u). The injected amount was 7–10 fmol.

4. Conclusions

There are a number of major factors which contribute to high-performance CE–ESI-TOF-MS. Volumetric constraints regarding injection size or improperly chosen conditions for CE separations compromise the overall quality of separation and detection from the very beginning of the analysis. The choice of CE buffer and ionic strength always is a compromise between optimal CE or ESI operation. Significantly improved detection can be achieved with the microelectrospray source, however the majority of CE separations necessitate conditions which exclude the use of this source. The operating parameters of the TOF-MS system must be adjusted to minimize mass discrimination while still maintaining efficient transmission for a large range of m/z ratio ions. Data acquisition, work-up, and storage must be fast enough to allow for accurate monitoring of the narrow eluting peaks from the CE system. TOF-MS is the major candidate for sensitive and fast CE–MS analysis.

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