ELSEVIER

Journal of Chromatography A, 703 (1995) 667–692

JOURNAL OF CHROMATOGRAPHY A

Review

Capillary electrophoresis–mass spectrometry

Jianyi Cai, Jack Henion*

Analytical Toxicology, Cornell University, 927 Warren Drive, Ithaca, NY 14850, USA

Abstract

As an on-line separation method, capillary electrophoresis (CE)–mass spectrometry (MS) distinguishes analytes by both their differences in electrophoretic mobilities and structural information. CE–MS combines the advantages of CE and MS so that information on both high separation efficiency and molecular masses and/or fragmentation can be obtained in one analysis. During the past few years CE–MS has undergone significant development both in instrumentation and application. Several ionization methods have been used for CE–MS. These include electrospray ionization, ion spray or pneumatically assisted electrospray ionization, and continuous-flow fast atom bombardment. The direct coupling of CE to desorption MS has not yet been reported, although publications have appeared on the off-line coupling of CE with matrix-assisted laser desorption ionization and ^{252}Cf plasma desorption MS using fraction collection. Numerous new applications of CE–MS have been published in the areas of biological sciences, pharmaceutical and drug metabolism, and environmental analysis. The majority of applications of CE–MS have been in the field of biological and biochemical studies.

Several limitations associated with CE–MS have precluded the technique being widely accepted for routine analysis. The major limitation is its relatively poor concentration sensitivity. The concentration detection limits of currently available CE–MS instrumentation are too high for most real-world applications. Other drawbacks with CE–MS include the fluctuation in analyte migration time and limitations in electrolyte selections. Approaches to improve the concentration sensitivity of CE–MS include on-line preconcentration either by capillary isotachophoresis or chromatographic methods. Another solution to increasing the sensitivity of CE–MS is the development of alternative types of mass spectrometers which offer the potential for greater sensitivity, such as ion trap, Fourier transform ion cyclotron resonance, and time-of-flight (TOF) mass spectrometers. Coated capillaries are useful in improving separation efficiencies of biomolecules by minimizing their adsorption onto the CE capillary walls.

At present, CE–MS is still not generally considered for routine analysis mainly due to its limited concentration sensitivity. As a complementary separation method to LC–MS with extremely high efficiency, CE–MS has the potential for wide acceptance in the future. The popularity of CE–MS will continue to grow, as more sensitive MS instrumentation and CE–MS interface are developed.

Contents

1. Introduction and historical review 668
2. CE–MS interface overview 669
 2.1. Electrospray 670
  2.1.1. Sheath-flow interface 670

* Corresponding author.

0021-9673/95/$09.50 © 1995 Elsevier Science B.V. All rights reserved
SSDI 0021-9673(94)01178-8

2.1.2. Sheathless interface ... 671
2.1.3. Comparison of sheath-flow and sheathless configurations ... 673
2.2. Ion spray ... 673
2.2.1. Liquid junction interface ... 673
2.2.2. Sheath-flow interface ... 674
2.2.3. Comparison of liquid junction and sheath-flow configurations ... 674
2.3. Continuous-flow fast atom bombardment ... 674
2.3.1. Liquid junction interface ... 675
2.3.2. Sheath-flow interface ... 675
2.3.3. Comparison of liquid junction and sheath-flow configurations ... 676
2.4. Comparison of electrospray and ion spray with continuous-flow fast atom bombardment ... 676
2.5. Other approaches ... 676
2.5.1. Other interfaces ... 676
2.5.2. Off-line CE–MS ... 677
3. Applications ... 678
3.1. Biologically important components ... 678
3.1.1. Peptides ... 678
3.1.2. Proteins ... 678
3.1.3. Enzymatic digests ... 679
3.1.4. Other biopolymers ... 679
3.1.5. Non-covalent binding study ... 679
3.2. Pharmaceutical and drug metabolism ... 679
3.2.1. Drugs ... 679
3.2.2. Drug metabolites ... 681
3.3. Environmental ... 682
3.4. Other applications ... 683
4. Limitations and solutions ... 683
4.1. Limitations ... 683
4.2. Analytical methodology improvement ... 684
4.2.1. On-line preconcentration by capillary isotachophoresis ... 684
4.2.2. Chromatographic on-line preconcentration ... 685
4.2.3. Other analytical approaches ... 686
4.3. Instrumental developments ... 686
4.3.1. Coated capillaries ... 686
4.3.2. Trapped ion mass spectrometers ... 687
4.3.3. Time of flight ... 688
4.3.4. Use of an array detector ... 688
Acknowledgements ... 688
References ... 688

1. Introduction and historical review

Microscale separation techniques, including high-performance capillary liquid chromatography and capillary electrophoresis have a number of practical advantages over conventional-scale analytical separation methods. Among them are high separation efficiency, high speed, and economy of sample size. High-performance capillary electrophoresis (HPCE) is an important microseparation technique in life sciences, as well as biotechnology and environmental research areas. Unlike high-performance liquid chromatography (HPLC), in which separation is due to the partition of solutes between the mobile phase and stationary phase, separation by CE is based on the difference in charge-to-mass ratio of the analytes. Therefore, a totally different selectivity is expected for the analytes providing a complementary separation method to HPLC.

Since the first demonstration of high separation efficiency with CE [1], the technique has significantly advanced. CE exhibits unparalleled resolving power for condensed-phase separations. It is advantageous in solving problems

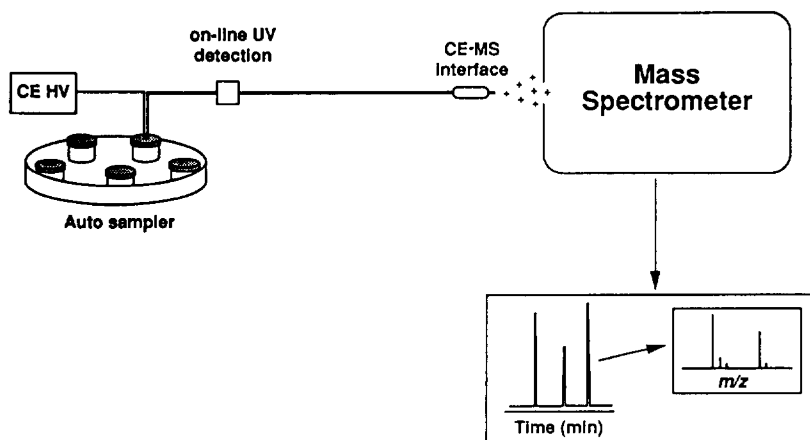

Fig. 1. Generic illustration of a CE–MS instrumental arrangement. HV = High voltage.

where high separation efficiencies are required in the case of analyzing very complex biological mixtures and where the sample amount is limited. An extreme case is the analysis of the contents of a single cell [2]. However, the limited loadability of CE also poses high demands on the detector sensitivity. This has led to a great deal of effort towards the development and improvement of CE instrumentation. A number of commercial CE instruments have become available. As a result, CE continues to attract interest. The subject has been reviewed extensively by a number of authors. The most recent comprehensive review summarizes the latest development and applications of the technique [3].

As new applications of CE continue to appear, the advantages and importance of CE in conjunction with mass spectrometry (MS) also become better appreciated. Analytical chemists are faced with the challenge of increasing sample complexity and decreasing sample quantities. Because of the complexity observed with most biological mixtures, there continues to be a need for the development of a highly efficient separation technique in conjunction with a sensitive and specific detector. The low quantities of analytes often available require nanoseparation techniques. The mass spectrometer is a selective and broadly applicable detector for analytical separations. It can provide information regarding the structure of unknown components present in a sample mixture with high specificity and sensitivity. The coupling of CE with MS combines the extremely high resolving power and structural information in one system. Like any other coupled separation technique such as GC–MS and LC–MS, the principal advantage of CE–MS is that analytes are identified both by their differential separation and their molecular masses and/or fragmentation patterns. An analytical separation that precedes MS analysis is often necessary to assure correct interpretation of the mass spectral data.

Since the first demonstration of the coupled technique [4], CE–MS has undergone significant development both in instrumentation and applications. A schematic diagram of CE–MS instrumental arrangement is shown in Fig. 1. Several reviews on the coupling of CE and MS have appeared [5–7]. A large number of new publications on the subject have emerged since the last review. While this review is intended to be comprehensive, the papers cited here will be on the basis of publication date with special emphasis on those appeared during the last two years.

2. CE–MS interface overview

From an MS perspective, the combination of CE and MS has relied on interfaces to allow

efficient transfer of analytes on-line from the electrophoretic capillary to the mass spectrometer without sacrificing separation efficiency. CE by its nature is particularly well suited to the separation of polar compounds readily ionizable in solution. These types of molecules have posed a challenge to conventional ionization techniques such as electron and chemical ionization due to thermal decomposition of non-volatile or thermally labile compounds. Over the last decade, several new ionization methods have been developed including fast atom bombardment (FAB), thermospray (TSP), atmospheric pressure ionization (API) methods, plasma desorption (PD) and matrix-assisted laser desorption ionization (MALDI). All of them except TSP are capable of producing ions from the condensed phase without high temperatures. There are several interfaces based on the API design, including electrospray (ESI), pneumatically assisted electrospray or ion spray (ISP), and the heated pneumatic nebulizer (HPN).

CE–MS requires the direct coupling of the ionization method to the liquid-phase separation techniques to allow MS detection. During the past few years, improvements have been made in interface reliability and reproducibility. The ionization techniques which have been successfully used for CE–MS include continuous-flow FAB (CF-FAB), ESI and pneumatically assisted electrospray or ISP. Desorption methods such as MALDI and PD have also been investigated although no direct on-line CE–MS results with these ionization methods have been reported to-date.

2.1. Electrospray

In ESI, ions may exist in solution as protonated molecules or adducts such as sodium and ammonium adducts in the positive-ion mode or deprotonated molecules in the negative-ion mode. According to Kebarle and Tang [8] there are four major processes involved in ESI-MS. Charged droplets are formed at the ESI capillary tip by an ion separation mechanism. These charged droplets are rapidly reduced in size by solvent evaporation and repeated droplet disintegration resulting in extremely small and highly charged droplets capable of forming gas-phase ions. Two different mechanisms have been proposed to account for the gas-phase ion production. Iribarne and Thomson [9] describe this process as ion evaporation where gas-phase ions are "evaporated" from the highly charged droplets. Schmelzeisen-Redeker et al. [10] suggested a desolvation mechanism in which single ions are released from very small droplets by solvent evaporation. Finally, these gas-phase ions undergo secondary processes by which they are modified in the atmospheric and sampling regions of the mass spectrometer.

ESI was first demonstrated by Zeleny in 1917 [11]. However, the recent breakthrough reported by Fenn and co-workers [12,13] reported the observation of multiply charged species with an ESI interface. This finding has revolutionized the applicability of conventional mass analyzers of limited mass-to-charge range to molecular mass determination and amino acid sequencing in protein biochemical investigations [14]. ESI has been successfully used for the coupling of separation techniques such as LC and CE. So far, two types of ESI interfaces known as sheath-flow and sheathless interfaces have gained general acceptance for CE–ESI-MS.

2.1.1. Sheath-flow interface

The first successful coupling of CE with MS was reported by the research group of Olivares et al. [4]. In this initial work, the cathode end of the CE capillary terminated within a stainless-steel capillary sheath where electrical contact was made, thus completing the CE circuit and initiating the electrospray. While no sheath liquid was used in this first report, there was a relatively large dead volume at the capillary terminus. An improved version of electrospray CE–MS interface was developed by the same research group where the metal contact at the CE terminus was replaced with a thin sheath of liquid flow [15]. A schematic diagram of ESI sheath-flow interface is shown in Fig. 2A. Since the CE capillary extended to the tip of the ESI interface and no additional mixing volumes and metal surface were involved, the sheath-flow

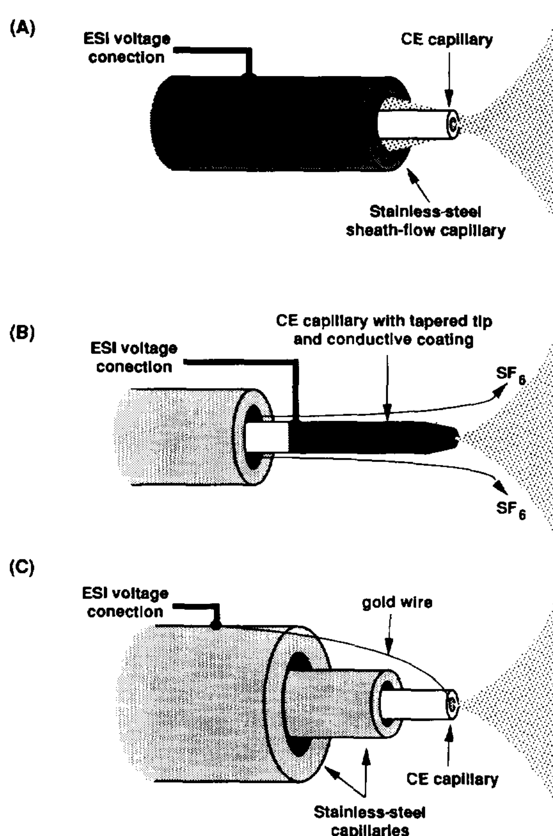

Fig. 2. Schematic diagram of CE–ESI-MS interfaces. (A) Coaxial sheath-flow configuration, (B) sheathless interface with tapered and conductive coated CE capillary, and (C) sheathless interface using a gold wire electrode.

interface provided both good sensitivity and separation efficiency. This system has been studied for better understanding and utilization [16–18]. A significant number of applications of CE–MS using electrospray ionization with a sheath-flow interface have appeared over the past three years, including applications in biological samples [19–21], drug metabolism [22–24] and impurity determination [25,26]. So far, the sheath-flow configuration has been the most widely accepted interface for CE–ESI-MS applications.

Perkins et al. [27] reported a comparison study between CE and nanoscale capillary liquid chromatography (ncLC) in conjunction with MS via a sheath-flow ESI interface. CE–ESI-MS was found to be more rapid and to provide better absolute sensitivity, while ncLC–ESI-MS provided promise for the analysis of dilute samples. In a similar study [28], CE–ESI-MS showed improved peak shapes while ncLC–ESI-MS gave better chromatographic resolution and was less susceptible to sample overloading. Both studies concluded that the two techniques are complementary.

One of the disadvantages associated with the use of sheath liquid is the unavoidable addition of ionic and neutral species in the sheath-flow which compete for available charge in the ESI process, thus lowering the maximum sensitivity obtainable [29]. In addition, difficulties have been experienced with these types of sources for obtaining a stable electrospray for extended periods of time, especially with a water sheath. In addition, studies by Karger and co-workers [30,31] have shown that the migration times of analytes in a CE–ESI-MS separation can be affected by sheath liquid composition. Due to the potential gradient across the CE capillary, anions from both the sheath-flow and background electrolyte migrate towards the anode. When the anions differ in the two solutions, a moving ionic boundary is formed inside the CE capillary. Since the pH may be different within this moving boundary, the electroosmotic flow-rates, the effective charge on the analytes and their migration rates will change once they enter the boundary. The sheath-flow composition was found to have changed the migration order of the analytes, as is shown in Fig. 3 [31].

2.1.2. Sheathless interface

A sheathless configuration for a particular ESI interface was developed for the characterization of proteins in aqueous solutions [32]. This early version of sheathless source was constructed from a stainless-steel syringe needle electropolished to a tapered tip to provide a stable electrospray for the characterization of proteins in aqueous solution without pneumatic nebulization. Since the onset potential required to obtain a stable electrospray decreases with a reduction in the O.D. of the sprayer needle, a sharp capillary tip allows the spray of aqueous solutions before the onset of a corona discharge. The

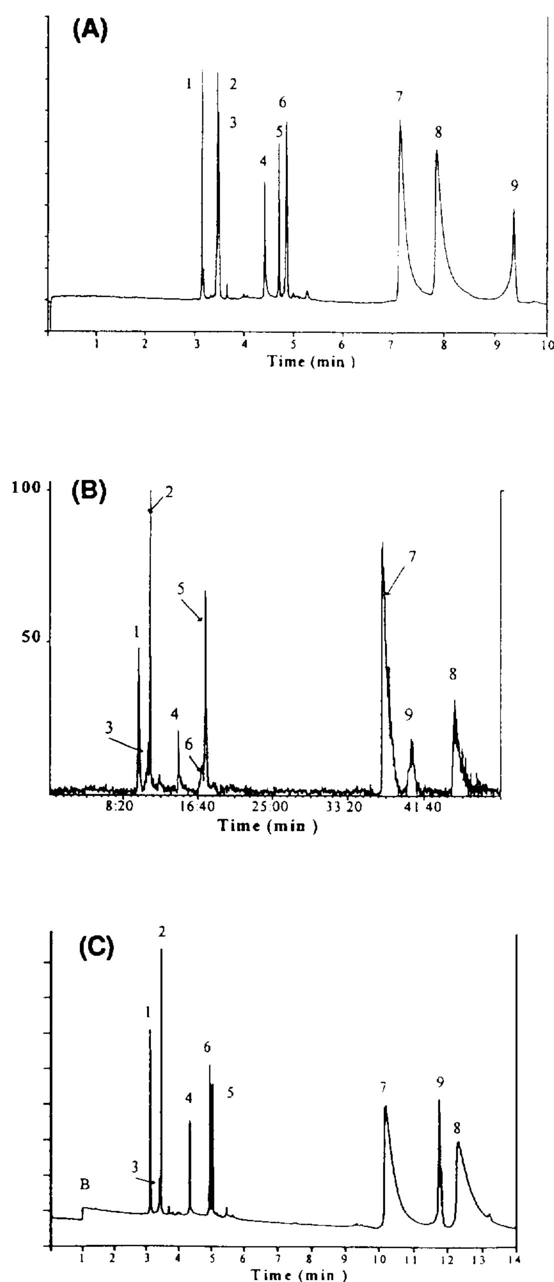

Fig. 3. Effect of liquid sheath on the separation of proteins by CE–ESI-MS. (A) CE–UV (214 nm) with no sheath flow; (B) CE–ESI-MS with 1% acetic acid in methanol–water (50:50) as sheath flow; (C) CE–UV (214 nm) with 1% acetic acid in methanol–water (50:50) as sheath flow. Peaks: 1 = lysozyme; 2 = cytochrome *c*; 3 = aprotinin; 4 = ribonuclease A; 5 = myoglobin; 6 = α-chymotrypsinogen A; 7 = β-lactoglobulin B; 8 = β-lactoglobulin A; 9 = carbonic anhydrase. From Ref. [31].

concept was adopted by Gale and Smith [29] who used a small-I.D. fused-silica capillary with an etched tip instead of the stainless steel needle for a pressure-infusion ESI source. A coaxial sheath gas of sulfur hexafluoride (SF_6) was applied to suppress the corona discharge at the sprayer tip.

A similar sheathless interface was adapted for CE–ESI-MS applications [33]. The sheathless design used a gold conductive coating at the CE capillary terminus to establish electrical contact with the CE effluent. Such a sheathless interface is illustrated in Fig. 2B. A publication by the same group [34] demonstrated the separation of a tryptic digest using the sheathless interface and a 10 μm I.D. CE capillary. The electrical contact was made by applying a silver conductive coating to the capillary terminus. The interface was found to produce and maintain a stable electrospray signal during a CE–ESI-MS experiment. The silver coating at the CE capillary terminus caused the formation of additional peaks which were believed to be the doubly charged adduct ion, $(M+H+Ag)^{2+}$, a phenomenon that may make molecular mass determination more problematic. Another sheathless interface, the "pinhole design" was also briefly investigated [33]. A microhole was created in the CE capillary wall by short circuit using high voltage. The microhole was covered with gold coatings. Preliminary results showed good performance with this interface design.

A sheathless ESI interface was also developed and coupled to a time-of-flight mass spectrometer by Fang et al. [35]. The electrical contact was made by inserting a gold electrode into the outlet of the CE capillary. The electrode was connected to a needle assembly where voltage was applied for ESI which completed the CE circuit (see Fig. 2C). By removing the polyimide coating and sharpening the silica capillary tip, they were able to increase the sensitivity by a factor of 4 to 5. A stable electrospray current was observed using 90% water in the CE electrolyte, which makes it possible for the direct transfer of optimum CE separation condition to CE–ESI-MS. Recently, Kriger et al. [36] incorporated a sheathless interface into a CE–ESI-MS system on an ion

trap mass spectrometer. A 50 μm I.D. fused-silica capillary was tapered to 45° angle at the exit tip and then gold-plated. The gold coating was prepared with a proprietary process and demonstrated excellent physical and chemical stability. A good electrospray was obtained with a wide range of CE electrolytes, including 100% aqueous solutions and solutions of high ionic strength. Using the sheathless interface they were able to demonstrate a detection limit for leucine-enkephalin in the total ion electropherograms on the order of 20 fmol and 10 fmol in the extracted ion current profile, indicating the improvement in sensitivity without the use of sheath-flow.

2.1.3. Comparison of sheath-flow and sheathless configurations

Gale and Smith [29] compared the performance of the two ESI interfaces using pressure infusion. Their study showed several advantages of the sheathless version, including great sensitivity, low required flow-rates and long-term stability. In another study [33], the performance of a gold-coated sheathless interface was compared with that of a sheath-flow interface regarding their dependence on buffer system and concentration, as well as capillary I.D. The sheathless interface turned out to offer better analyte detectability. It was found that the sheathless configuration also eliminated the possible interferences from the sheath solvents such as charge state distribution shift [36].

2.2. Ion spray

ISP is closely related to ESI, the difference being the application of a nebulizing gas which permits stable electrospray operation at flow-rates up to 1 ml/min [37] whereas pure electrospray has been restricted to flow-rate below 10 μl/min [13]. Ikonomou et al. [38] have published a comprehensive study on the comparison of the mechanisms and performance of ESI and ISP. Since the nebulization of the solvent for ESI is not dependent on the electrohydrodynamic instability at the capillary tip, ISP is able to produce charged droplets and mass spectra even when the capillary voltage is below that required for the onset of electrospray [38]. In addition, ISP provides superior sensitivity and signal stability at higher flow-rates as compared with pure ESI. ISP provides a mild ionization via an ion evaporation process which results in primarily molecular mass information in the form of singly and multiply charged ions. In fact, the mass spectra of basic compounds are essentially the same with either ESI or ISP [38]. To-date, two types of CE–MS coupling have been described with ISP interfaces: the liquid junction and coaxial sheath-flow configurations.

2.2.1. Liquid junction interface

A liquid junction design was developed by Henion and co-workers [39,40] for the coupling of CE to MS using the pneumatically assisted electrospray approach. The liquid junction was constructed from a stainless-steel tee. The cathode end of the CE capillary and the end of the ISP needle were positioned in the center of the tee opposite each other with a gap of 10 to 25 μm which could be adjusted by tightening or loosening the capillary fittings. The top opening in the tee was fitted with a make-up liquid reservoir while the bottom opening was used for alignment and adjustment of the gap between the CE capillary and the ISP needle. A schematic diagram of the liquid junction interface is shown in Fig. 4A. The interface was found to compensate for the different flow-rates required by the ISP and the CE.

The utility of the system was demonstrated by applications including the determination of a synthetic mixtures of pesticide [39], peptide standards and tryptic digests [40,41], sulfonylurea herbicides from soil [42], sulfonated azo dyes in waste water extracts [43], synthetic drug mixtures and drug residues from human urine [44]. MS detection was achieved in either the positive- or negative-ion modes. Separation efficiencies as high as 300 000 theoretical plates were obtained indicating negligible peak broadening due the interface [39]. In addition, the liquid junction interface was successfully used for the coupling of gel-filled CE to MS [45].

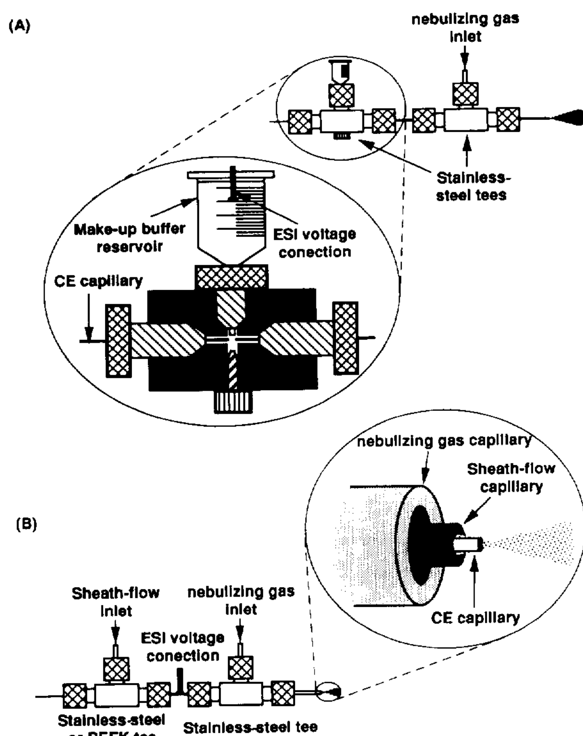

Fig. 4. Schematic diagram of CE–ISP-MS interfaces. (A) Liquid junction and (B) coaxial sheath-flow configurations.

2.2.2. Sheath-flow interface

The ISP sheath-flow interface is similar to that described for the ESI system with the difference being the addition of a coaxial nebulizing gas as is shown in Fig. 4B. It has been described by Thibault and co-workers [46,47]. Later, Pleasence et al. [48] modified a commercial ISP interface so that both the liquid junction and coaxial approaches could be used. Henion et al. [49] have modified a coaxial sheath-flow ISP interface for the coupling of CE to a benchtop ion trap mass spectrometer. The interface showed excellent stability and sensitivity. Using finger-tight fittings, the interface allows easy exchange of the CE capillary when necessary. More recently, Tetler et al. [50] have studied the influence of the dimensions of the capillaries in a coaxial CE–MS interface on the stability and sensitivity of the systems.

ISP using the coaxial sheath-flow configuration has been used in numerous applications. Among them are the separation and characterization of peptides and proteins [47,51–53], drug–protein conjugates [54], drug metabolites [55], natural products [49], inorganic materials [56,57], textile dyes [58] and degradation products of chemical warfare agents [59]. CE–ISP-MS has also been applied to the areas of environmental importance such as the analysis of paralytic shellfish poisons in sea food [46,60], and the determination of agrochemicals and industrial diisocyanates [61].

2.2.3. Comparison of liquid junction and sheath-flow configurations

Pleasance et al. [48] reported a comparison study between the liquid junction and coaxial interfaces with regard to ruggedness, ease of use, sensitivity, and electrophoretic performance. A modified commercial ISP interface was constructed which allowed the use of both liquid junction and sheath-flow configurations on the same source. They found that both interface designs were capable of providing efficient coupling of CE to MS with the coaxial sheath-flow configuration being more robust and reproducible. An additional advantage with the sheath-flow interface is that it provides the potential for flow injection analysis since the make-up flow is delivered independently of the CE effluent. While the liquid junction CE–MS coupling approach provided improved sensitivity and ion current stability when properly assembled, the optimum setup of this system was more challenging than that of the sheath-flow system.

2.3. Continuous-flow fast atom bombardment

FAB was first introduced by Barber et al. [62]. This technique dramatically extended the capability of MS in the early 1980s for the determination of fragile and polar compounds. FAB was considered a static analytical technique until Ito et al. [63] reported continuous-flow (CF) FAB. The combination of CE with CF-FAB-MS was first demonstrated by Minard et al. [64]. Since then there has been a great deal of effort to improve its performance. The low flow-rates required to maintain the high efficiencies of CE

are incompatible with the typical CF-FAB flow-rates, which require a typical flow-rate of 5 μl/min. To circumvent this problem, interfaces using sheath-flow or liquid junction configurations have been introduced to couple CE to CF-FAB.

2.3.1. Liquid junction interface

The first CE–CF-FAB-MS work was performed using a liquid junction interface [64]. Its construction involved alignment of the CE capillary and the FAB flow capillary. A 20-μm wide junction between the two capillaries was carefully adjusted and immersed in the FAB matrix containing methanol or methanol–acetonitrile with 4 to 20% glycerol. This interface provided relatively low separation efficiency. Only 6800 theoretical plates were obtained for the separation of a synthetic peptide mixture. Reinhoud et al. [65] have studied the performance of a similar liquid junction interface in terms of peak broadening and found that although the liquid junction resulted in some loss of resolution in separation, plate numbers over 10 000 were achievable. They demonstrated the concentration detection limit of 1 ng/ml of dextromethorphan. The liquid junction interface has been redesigned to allow easy mounting and alignment of the CE and flow FAB capillaries, as well as the inter-capillary gap adjustment [66]. The interface was further modified by Caprioli et al. [67], who reported CE–CF-FAB-MS and liquid junction interface for the analysis of synthetic mixtures of peptides and protein digests. A liquid junction-like interface was constructed and was used for the identification of deoxynucleoside–polyaromatic hydrocarbon adducts [68]. While similar to the early version in certain respects, it contained several unique features including the assembly which consisted of two Nalgene tees joined by a glass capillary flame-drawn to a suitable I.D. to provide a snug alignment for the CE and FAB capillaries. The liquid junction interface for CE–CF-FAB-MS is very similar to those for CE–ISP-MS with the exception of the absence of nebulizing gas as is shown in Fig. 5A.

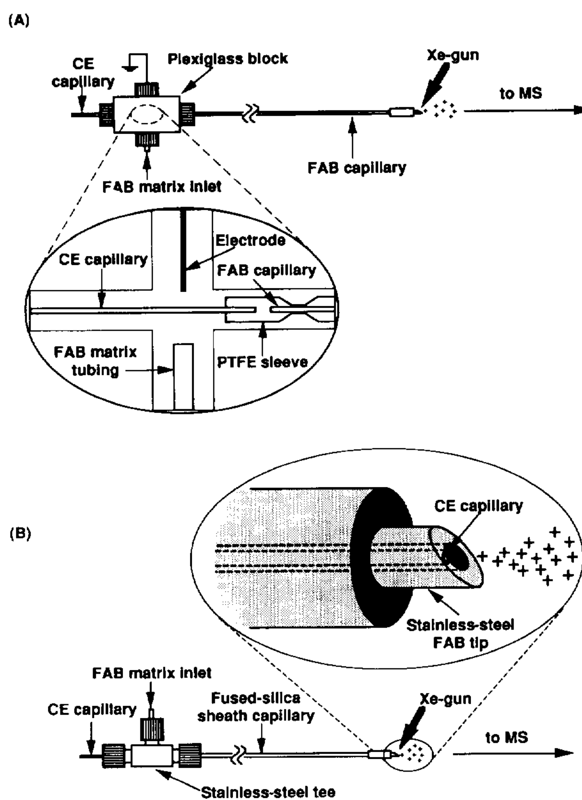

Fig. 5. Schematic diagram of CE–CF-FAB-MS interfaces. (A) Liquid junction and (B) coaxial sheath-flow configurations.

2.3.2. Sheath-flow interface

The coaxial sheath-flow interface is another approach to couple CE and CF-FAB-MS, which was first designed for the coupling of open tubular liquid chromatography to CF-FAB [69]. The interface was adapted for CE–CF-FAB-MS analysis [70,71]. The coaxial configuration allows independent optimization of the composition and flow-rates of CE effluents and the FAB matrix, minimal band broadening and precludes any deleterious effects of the polar, viscous FAB matrix on the CE separation process. The sheath-flow interface for CE–CF-FAB-MS is almost identical to those for CE–ESI-MS with the exception of sheath flow being a FAB matrix solution. A schematic representation of sheath flow CE–CF-FAB-MS interface is shown in Fig. 5B. These initial demonstrations of on-line CE–CF-FAB-MS using coaxial sheath-flow interface

showed the separation of peptide mixtures with separation efficiencies of several hundred thousand theoretical plates with limits of detection of less than 10 fmol [71]. In order to minimize the vacuum induced flow, a small-I.D. (13 μm) CE capillary was used with the end being withdrawn several mm back into the sheath capillary (see Fig. 5B). The sheath-flow interface was used for the characterization of peptide and protein mixtures, as well as protein digests [72,73].

Comparisons between CE and ncLC in conjunction with CF-FAB-MS have been reported [74,75]. These findings suggest that the analysis time is significantly shorter for CE than for cLC. The detection limit by CE in terms of absolute amount injected is several orders of magnitude higher than for cLC. The two separations also gave different elution order of the same analytes. The main disadvantage of CE is that overloading occurs at much lower levels than for cLC.

2.3.3. Comparison of liquid junction and sheath-flow configurations

The two interface configurations for CE–CF-FAB-MS have been compared in terms of performance and ruggedness [76]. The advantages of a coaxial interface include no dead volume and higher theoretical plate numbers. However, the coaxial interface is difficult to handle and set up due to the greater manipulation of relatively brittle capillaries and the problems associated with high voltage arcing through the thin capillary wall. In addition, ion source vacuum-induced parabolic flow present in the CE capillary resulted in shorter electrophoresis times and loss of CE performance. Only small-I.D. capillaries have been used to minimize the effect associated with the mass spectrometer vacuum. The vacuum-induced flow-rates in a coaxial interface were found to be 4 μl/min for a 1 m × 75 μm I.D. CE capillary and 3 nl/min for an equal length of capillary with 13 μm I.D. [70]. The liquid junction interface was found to offer equal or greater overall separation efficiencies. It was relatively easy to set up and operate, and allows larger-I.D. capillaries to be used to improve the sample load. The disadvantages of liquid junction interface are the large dead volume and lower overall sensitivity. Both interfaces were found to have degraded a significant degree of CE performance.

2.4. Comparison of electrospray and ion spray with continuous-flow fast atom bombardment

Several research groups have compared the two ionization methods with regard to the performance of CE–MS [74,77]. There are several attractive features of CE–MS using the ESI interface. Since larger-I.D. capillaries can be used for the ESI interface, the loading capacity should be greater than those using the coaxial sheath-flow CF-FAB interface where only small-I.D. capillaries (ca. 10 μm) can be used due to the pressure drop at the source region which lead to hydrodynamic flow within the CE capillary distorting the plug flow of electroosmotic flow and causing band broadening. The ESI interface also gives reduced background noise and higher sensitivity [78]. In the study conducted by Nichols et al. [77], a micro liquid junction configuration was used for both CF-FAB and ISP interfaces for the investigation of the same analytes which included aromatic sulfonates, quaternary amines and a synthetic peptide mixture. The two analytical approaches were found to provide comparable sensitivity and analytical information. However, CF-FAB provided greater peak broadening (see Fig. 6). Both systems were considered as less than routine with respect to analytical ruggedness.

2.5. Other approaches

2.5.1. Other interfaces

A microflow ultrasonic nebulizer interface was developed by Tarr et al. [79]. The nebulizer was able to provide stable nebulization and essentially 100% transport efficiency at flow-rates of 5 to 20 μl/min suggesting good potential for application in interfacing CE and microcolumn LC to MS. However, no actual CE–MS experiments have been conducted with this interface.

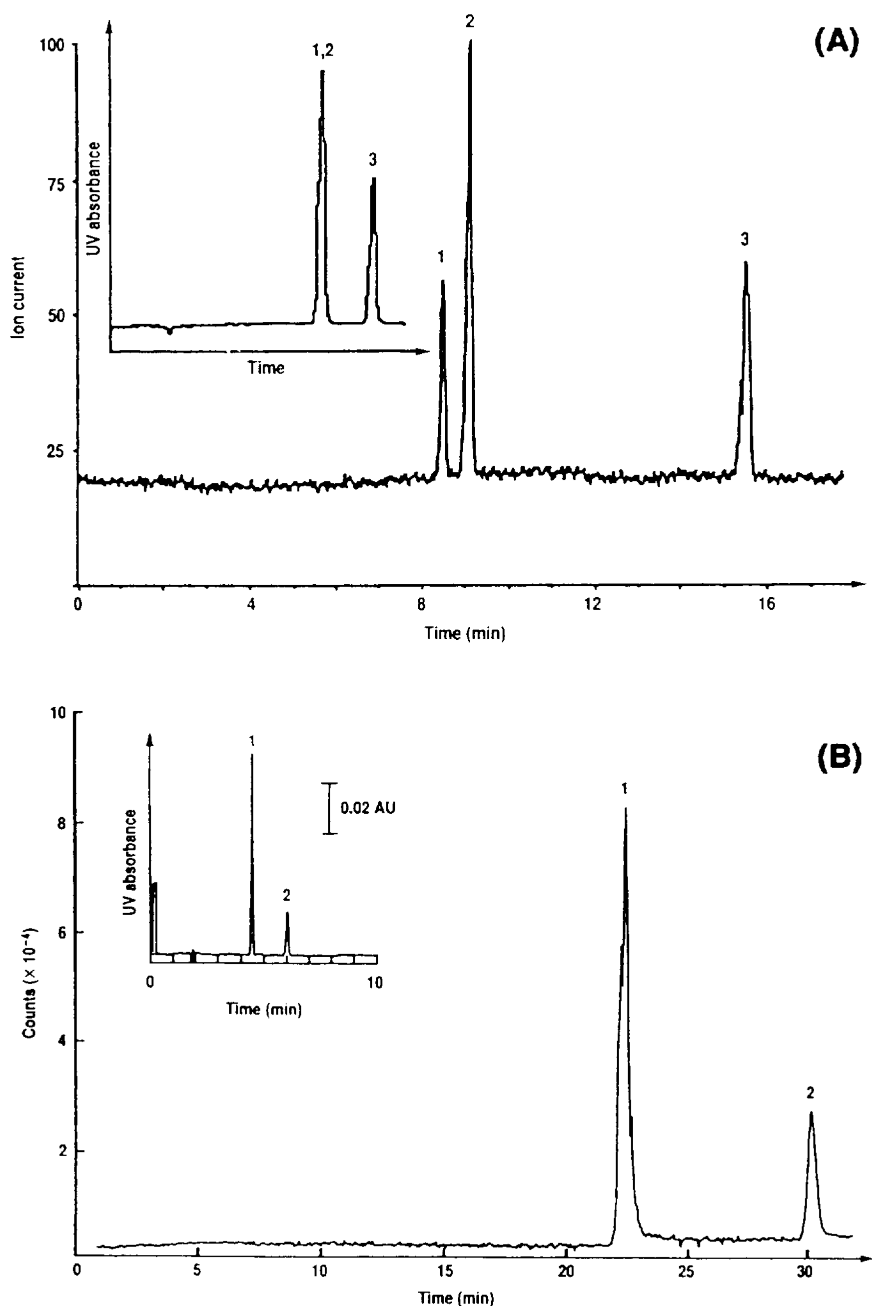

Fig. 6. Total selected ion current and CE–UV (inset) electropherograms of a sulfonated aromatic mixture obtained by (A) CE–ISP-SIM-MS and (B) CE–CF-FAB-SIM-MS, using liquid junction interfaces. From Ref. [77].

2.5.2. *Off-line CE–MS*

While on-line methods are preferred, they are often not available to interested practitioners. Ionization by desorption methods such as PD and MALDI are very useful for the characterization of large molecules. Molecular masses up to several hundred kilodaltons can be determined by MALDI-MS by irradiation of small sample

spots [80]. However, on-line CE coupling of desorption MS is not yet been reported. Takigiku et al. [81] described the use of a "porous glass joint" near the cathodic end of the CE column to collect fractions for desorption MS. Off-line coupling of CE with laser desorption MS was used by Keough et al. [82] for the determination of proteins. Weinmann and co-workers [83,84] have developed a method using nitrocellulose-coated targets for the isolation and transfer of polypeptides and proteins from CE separation for subsequent characterization by ^{252}Cf PD-MS. Several modes of off-line coupling of CE–MALDI-MS have been investigated by Van Veelen et al. [85]. The best performance was obtained using a moving belt-like system in a stepwise manner and a sheath-flow of matrix solution followed by scanning of laser desorption target. Tomer et al. [86] have recently described methodologies for combining CE and affinity chromatography off-line with MALDI-TOF-MS. Using a coaxial sheath-flow to avoid memory effects, CE fractions were collected and then analyzed by MALDI-TOF-MS. Another approach was described by Blakley et al. [87], where a computer-controlled x–y table and an effluent transfer tube were employed for fraction collection and MALDI-TOF-MS analysis. A method for fraction collection from an automated micellar electrokinetic capillary chromatographic system has been described [88]. Aliquots of 5 μl of fractions were collected with recoveries of more than 75%. However, the ion suppression effect of sodium dodecyl sulfate present in the buffer prohibited the direct analysis by FAB-MS.

3. Applications

3.1. Biologically important components

One of the advantages of incorporating ESI-MS and ISP-MS with the determination of biological compounds with high molecular masses lies in the multiple charging of the analytes that can occur under ESI conditions. The majority of applications of CE–MS have been in the field of biological and biochemical studies.

3.1.1. Peptides

A number of reports have appeared on the characterization of synthetic mixtures of peptides and proteins by CE–MS [17,41,89]. A CE–ESI-MS system was optimized for the analysis of peptide mixtures [20]. Full-scan mass spectra were acquired using 160 fmol of peptide standards loaded on-column. A synthetic mixture of six dipeptides was examined by CE–ISP-MS [51]. Standard peptide mixtures have also been characterized by CE–ESI-MS on an ion trap [90] and TOF [35] mass spectrometers. Rosnack et al. [91] discussed the capability of CE–ESI-MS in the analysis of synthetic peptide impurities, a practical problem encountered in a peptide synthesis laboratory. Recently, a synthetic 37-residue peptide fragment of a monoclonal antibody against *Herpes* virus was characterized by CE–ISP-MS and by off-line ^{252}Cf PD-TOF-MS [53]. Five impurities formed in the synthesis were separated and identified by CE–ISP-MS. Weinmann et al. [83] carried out structural characterization of polypeptides and proteins using off-line coupling of CE and ^{252}Cf PD-MS analysis. Tomlinson and co-workers [92,93] described on-line sample enrichment and/or desalting of peptides for subsequent determination by CE–ESI-MS using a precolumn packed with HPLC packing.

3.1.2. Proteins

The determination of recombinant bovine and porcine somatotropins (rbSt and rpSt) was reported by Tsuji et al. [19]. A detection limit of 100 fmol was obtained. The separation and determination of a synthetic protein mixture was presented by Cole et al. [94] using a hydrophilic derivatized CE capillary. Thompson and co-workers [30,31] studied the liquid sheath effects on the CE–ESI-MS separations of standard proteins in a coated capillary. The same authors have also investigated on-column transient isotachophoretic sample preconcentration to improve protein detection limits by CE–ESI-MS [95]. Wahl et al. [17] used the small-diameter-capillary approach to increase peptide and protein detection sensitivity in CE–ESI-MS. Volk et al. [52] demonstrated the rapid and efficient separation and characterization of closely related glycoforms of glycoproteins and structurally simi-

lar proteolytic fragments using CE–ISP-MS. A synthetic drug–protein conjugate mixture was analyzed by CE–ISP-MS [54]. Lysozyme and its conjugates with one to three naproxen molecules were separated and identified. CE–ESI-MS has been used for the analysis of a complex mixture of peptides and small proteins, snake venoms of Black Mamba [21]. Molecular mass and some sequence information for those toxins were obtained using CE-selected ion monitoring (SIM) MS and full-scan CE–MS analysis. Fig. 7 shows the CE–ESI-SIM-MS electropherograms of some peptides observed in the whole venom from Black Mamba [21].

3.1.3. Enzymatic digests

Tryptic digests have been characterized by a numbers of workers as model systems [41,74,96]. Separation of tryptic digests of bovine *Candida krusei* and equine cytochrome *c* has been reported using CE–ESI-MS and an aminopropylamine-treated capillary [34]. By using a 10 μm I.D. capillary, sample amounts in the 30 fmol region were loaded into the capillary. Tryptic digests of bovine, equine and tuna cytochrome *c* were characterized by CE–ESI-MS using a 20 μm I.D. capillary [33]. The total ion electropherograms obtained with these enzymatic digests are shown in Fig. 8. The chymotryptic digest of ubiquitin was studied by CE–ESI-MS using Fourier transform ion cyclotron resonance (FTICR) MS [97]. Takigiku et al. [98] presented characterization of protein digests by CE–ISP-MS. The hydrolysis of protein was carried out in a fused-silica capillary with immobilized trypsin (capillary microreactor). This technique may be advantageous for sequencing minute quantities of protein with minimal sample handling.

3.1.4. Other biopolymers

Separation and characterization of lipopolysaccharides from *B. catarrhalis* and *H. influenzae* type B has been demonstrated using CE–ISP-MS [99]. CE–ESI-MS was used for the analysis of the oligosaccharides isolated from glycoproteins [100]. Zhao et al. [101] presented the determination of a nucleotide and its radiation-damaged products by capillary isotachophoresis (cITP)–ESI-MS. Characterization of deoxynucleoside–polyaromatic hydrocarbon adducts by CE–CF-FAB-MS was demonstrated by Wolf et al. [68]. The adducts were formed by covalent attachment of four polycyclic aromatic hydrocarbon (PAH) and amino-PAH compounds to deoxyguanosine. Analysis of DNA–carcinogen adducts by CE–ESI-MS was investigated [102]. A reactive metabolite of benzo[*a*]pyrene, benzo[*a*]-pyrene-7,8-dihydrodiol 9,10-epoxide metabolite, was reacted with DNA in vitro to form adducts which were subsequently cleaved into individual nucleotides and analyzed by CE–ESI-MS.

3.1.5. Non-covalent binding study

CE–MS has been found to be a useful technique for the study of biomolecular non-covalent interactions because it combines separation, and characterization as well as quantitation in one operation. Recently, we have investigated the non-covalent binding of FK506 and rapamycin to FKBP by CE–ISP-MS [103]. The observed relative binding affinities of rapamycin and FK506 to FKBP is 9/1 which is consistent with the published K_i values of the two ligands. Goodlett et al. [104] studied the thermal stability of ribonuclease S, an enzymatically active non-covalent complex, by CE–ESI-MS. The intensity of RNase S molecular ion peaks were observed to decrease with the increasing temperatures at heated capillary–skimmer interface and the in the sample solution. Uzabiaga et al. [105] have recently presented a study on the non-covalent complex of human antithrombin III with the pentasaccharide SR 90107A by CE–ESI-MS. CE–ESI-MS was found to be superior to direct infusion.

3.2. Pharmaceutical and drug metabolism

3.2.1. Drugs

CE–MS is finding increasing applications in the analysis of sample for pharmaceuticals, therapeutic and xenobiotic metabolites. CE–ISP-MS was used for the determination of small drug molecules such as sulfonamides and benzodiazepines using the liquid junction interface [44]. The analysis of an extract of human urine of a person

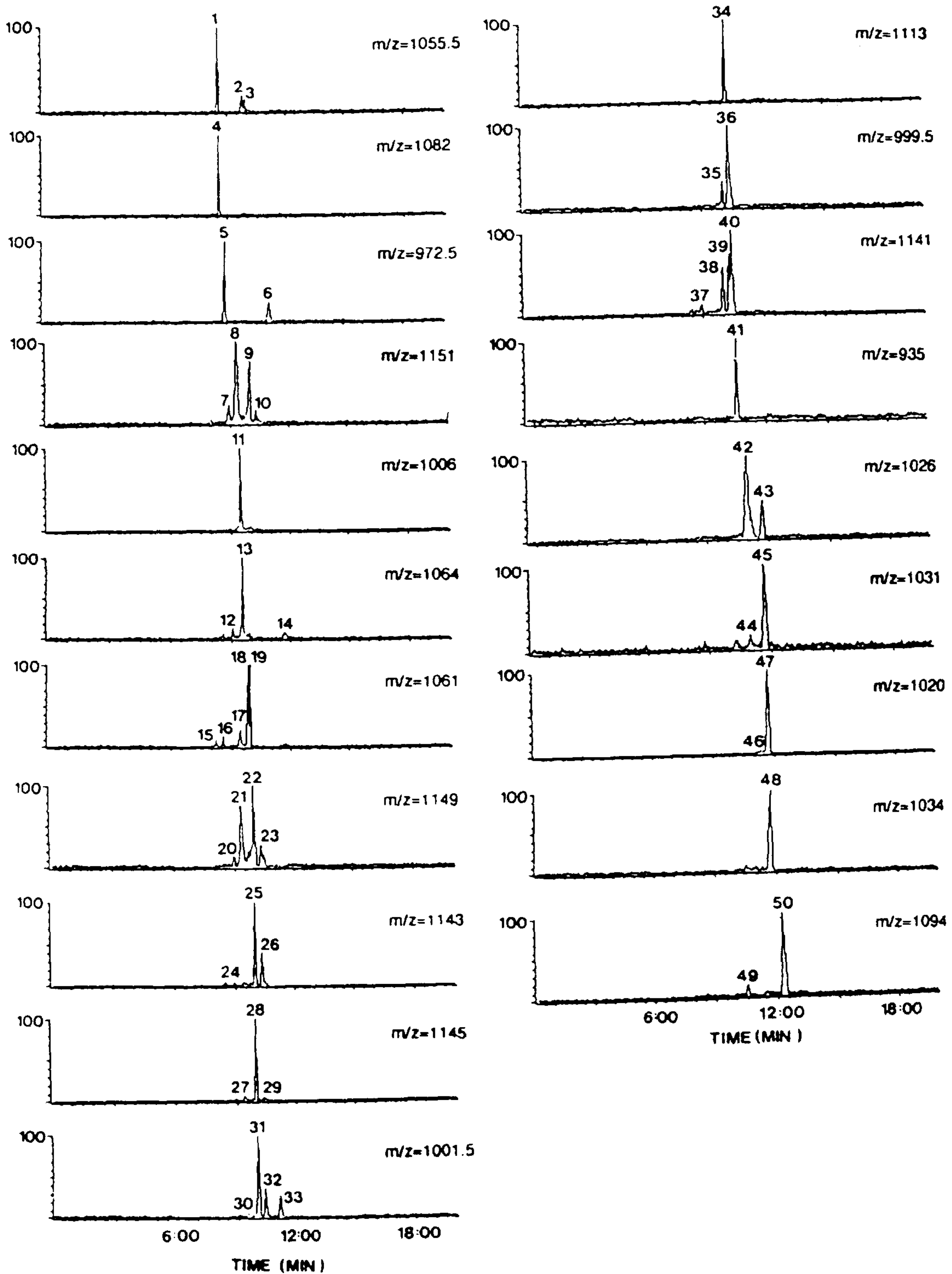

Fig. 7. CE–ESI-SIM-MS determination of peptides in the whole venom from *Dendroaspis polylepis polylepis*. From Ref. [21].

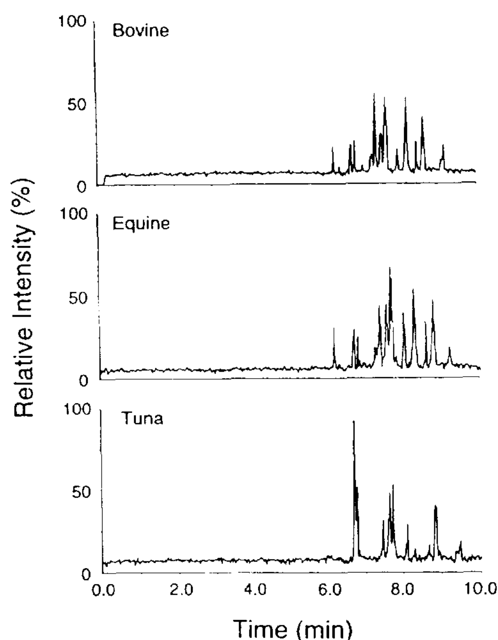

Fig. 8. Total ion electropherograms from tryptic digests of bovine, equine and tuna cytochrome *c* by CE–ESI-MS using a sheathless interface. From Ref. [33].

who had received an oral dose of flurazepam dihydrochloride was demonstrated. The separation of sulfonamide drugs was also reported using CE–ESI-MS [27,106]. Parker et al. [28] reported the analysis of macrolide antibiotics by nanoscale packed capillary LC and CE–ESI-MS. An application of CE–ISP-MS in the study of Chinese herbal medicine remedy in tree barks and Chinese herbal medicine tablets was published using a modified benchtop ion trap mass spectrometer [49,107]. Quantitative analysis was carried out using an internal standard.

Impurity determination is also very important in pharmaceutical preparations. A CE–ISP-MS system was evaluated for the detection and characterization of trace impurities in sample mixtures using model peptides and small drug molecules [108]. The characterization of chiral drugs has drawn increased attention in the pharmaceutical industry community [109]. Chiral separation by CE has been reviewed recently [110,111]. The separation and characterization of chiral drugs by CE–ISP-MS have been investigated in our research group [112]. Several synthetic chiral drug mixtures were studied using cyclodextrin as a chiral selector in the electrolyte. Both the protonated molecules of the enantiomers of terbutaline and their respective cyclodextrin–drug complex were shown in the CE–ISP-SIM-MS electropherograms (Fig. 9).

3.2.2. Drug metabolites

CE–MS is gaining popularity in drug metabolism studies. Tomlinson and co-workers [22–24,113,114] recently published their applications of CE–ESI-MS in drug metabolism with a particular emphasis on neuroleptic drugs. The use of a non-aqueous solution as electrolyte was emphasized due to the low solubility of the drugs in pure aqueous solutions. Methanol (100%) containing acetic acid and/or ammonium acetate was used as CE electrolyte [22,23,114]. Shown in Fig. 10 are the CE–ESI-SIM-MS electrophero-

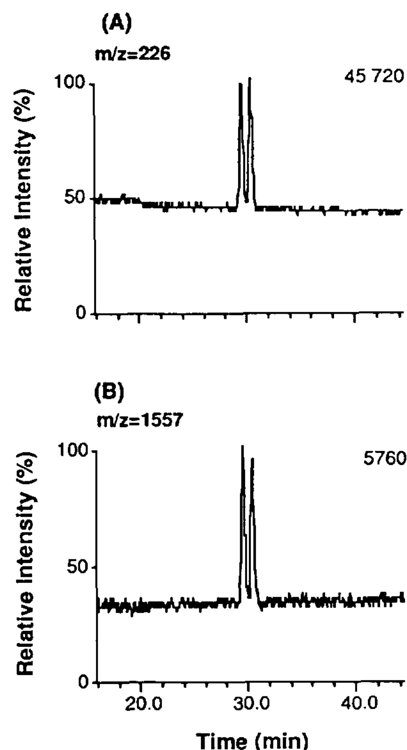

Fig. 9. CE–ISP-MS electropherograms showing the separation of (A) terbutaline enantiomers and (B) their respective cyclodextrin-drug complexes. From Ref. [112].

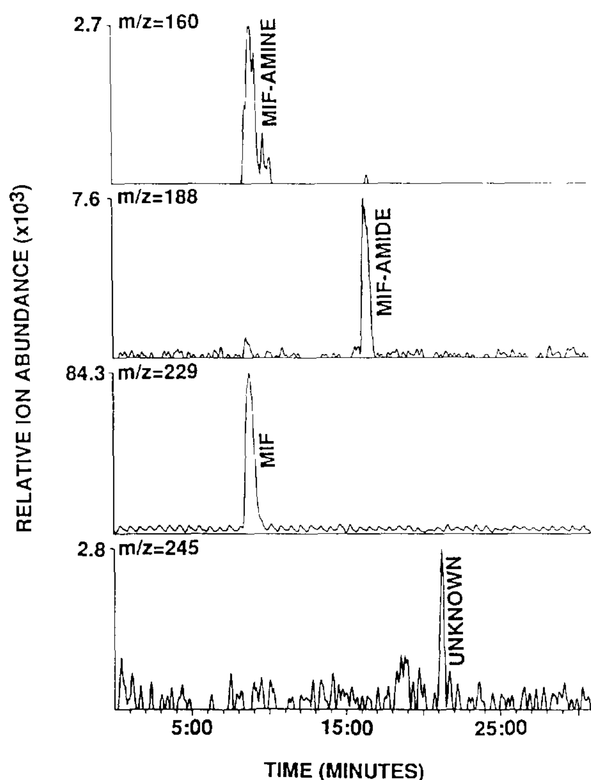

Fig. 10. CE–ESI-MS electropherograms for the analysis of a metabolite mixture derived form a rat hepatic microsomal incubation of Mifendine (MIF) using 5 m*M* ammonium acetate and 100 m*M* acetic acid in 100% MeOH as electrolyte. From Ref. [114].

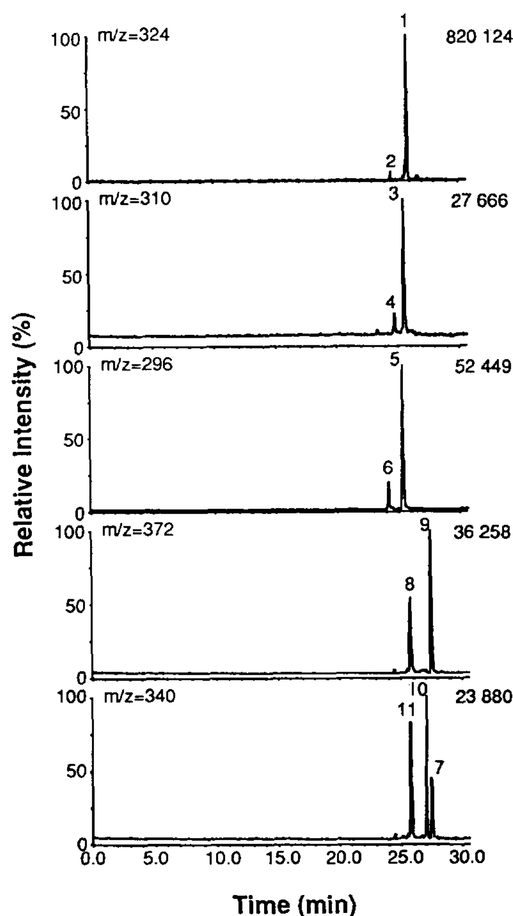

Fig. 11. CE–ISP-MS electropherograms for the identification of metabolites derived from human hepatic microsomal incubation of LSD. Peaks: 1 = LSD; 2 = iso-LSD; 3 = nor-LSD; 4 = iso-nor-LSD; 5 = lysergic acid ethylamide (LAE); 6 = iso-LAE; 7 = 2-oxo-LSD; 8–11 = unknown metabolites. From Ref. [55].

gram of a metabolite mixture derived from a rat hepatic microsomal incubation of Mifentidine. We have demonstrated the identification of metabolites of lysergic acid diethylamide (LSD) in human hepatic microsomal incubation by both LC–MS–MS and CE–MS–MS using a sheath-flow ISP interface [55]. CE–ISP-MS as a complementary separation tool greatly facilitated the identification of unknown metabolites. A representative electropherogram is shown in Fig. 11.

3.3. *Environmental*

CE–MS has been applied to the determination of compounds of environmental concern, such as agrochemicals, pesticides, inorganic compounds and dyes. A fast CE–ISP-MS determination of sulfonylurea crop-protection agents was demonstrated by Garcia and Henion [42]. Full-scan CE–ISP-MS collision-induced dissociation mass spectra were obtained from 35-pmol levels of standard injected. The analysis of ionic textile dyes using CE–ISP-MS and MS–MS was presented by Lee et al. [43]. In this early study, the presence of three sulfonated azo dyes in a waste water extract was confirmed by CE–ISP-MS–MS experiments. CE–ISP-MS has been used for the determination of quaternary ammonium herbicides [61]. The limit of detection at 5 ppm and linear calibration graphs were obtained with correlation coefficients above 0.995. The applica-

tion of CE–ISP-MS to the analysis of mixtures containing a variety of antibiotic classes used in the fish aquaculture industry has been reported by Pleasance et al. [48]. The presence of several antibiotics in shellfish extracts was confirmed at low ppm levels. Lamoree et al. [115] have demonstrated the application of CE–ESI-MS for the determination of β-agonists which are being used illegally in the cattle industry as repartitioning agents to increase meat production. By using on-capillary cITP for loadability enhancement, concentration detection limits in the ng/ml range were obtained. The determination of some β-agonists in spiked calf urine was demonstrated. Hines et al. [116] have evaluated a CE–ESI-MS system for the detection and quantification of Fumonsin B_1, the most abundant fumonisin in corn samples.

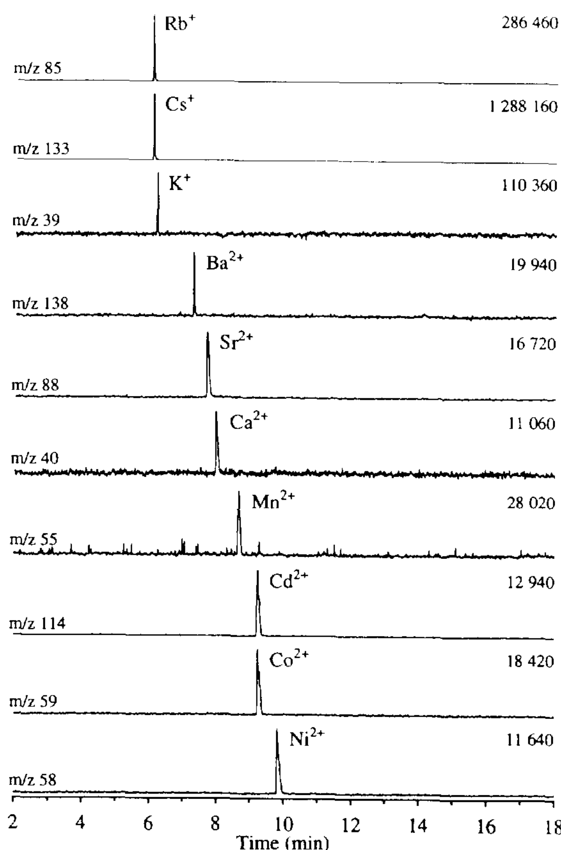

Fig. 12. CE–ISP-MS electropherograms of ten inorganic cations. From Ref. [57].

3.4. Other applications

Pleasance et al. [60] reported the application of CE–ISP-MS to the analysis of paralytic shellfish toxins isolated from marine matrices. The application of CE–ISP-MS to the determination of textile dyes has been reported [58,117]. A CE–ESI-MS system optimization for cationic and anionic laser dye analysis has been described using opposite polarities at the injector and interface [25]. The technique was employed to evaluate the purity of the laser dyes. Kostiainen et al. [59] have demonstrated the identification of degradation products of some chemical warfare agents such as substituted organophosphorus acids by CE–ISP-MS using the negative ionization mode. Quantitative analysis was performed with good linearity. Another application of CE–ISP-MS is the detection of inorganic ions [56,57]. Example electropherograms obtained by CE–ISP-SIM-MS are shown in Fig. 12 where ten inorganic cations are shown separated. The analysis of an aqueous acidic extract of used aircraft engine oil revealed high levels of lead as well as lower levels of other metal ions such as chromium and nickel [56].

4. Limitations and solutions

4.1. Limitations

Although numerous publications have appeared on CE–MS, this technique is still not widely accepted for routine use. The major limitation of CE is the limited sample volumes that can be analyzed without compromising separation efficiency. Consequently, the concentration detection limit for CE is usually several orders of magnitude higher than that of chromatographic methods. Using the currently available instrumentation, CE–MS detection limits for most applications are too high so that it seems unlikely for it to be used in routine analysis. For example, a study was conducted comparing the performance of CE–MS with that of microbore LC–MS in the determination of endogenous amounts of leucine-enkephalin and

methionine-enkephalin in equine cerebrospinal fluid using identical sample clean-up and enrichment procedures [118]. CE–MS was found to be limited in its concentration detection capacity owing to its much smaller injection volume.

Another drawback with CE–MS is that migration times tend to fluctuate with a change of temperature in the environment. Although some manufacturers have incorporated temperature-controlling system into their CE instruments, these devices cannot be effectively utilized in CE–MS applications because a large portion of the CE capillary is extended between the CE instrument and the mass spectrometer. For applications such as regulatory work or those involving unknown components in a mixture, the use of a suitable internal standard would be necessary [48,49]. The chemical condition of the CE capillary inner walls also plays an important role in CE separation. The reproducibility and ruggedness of CE–MS are not currently as good as those of LC–MS.

Like LC–MS, the use of non-volatile buffers in CE–MS is generally avoided. Compromises are often made in choosing appropriate operational conditions for CE–MS. Sensitivity limitations as well as ion source plugging problems created by the use of non-volatile buffers have impeded the direct transfer of CE separation conditions to on-line CE–MS. Non-volatile additives such as cyclodextrins are widely used in the separation of closely related analytes including optical isomers [110–112]. However, the concentrations of these additives are often limited by practical restrictions [26,119]. The MS sensitivity may deteriorate as the bulk flow of surfactant enters the mass spectrometer source region.

4.2. Analytical methodology improvement

4.2.1. On-line preconcentration by capillary isotachophoresis

A number of techniques have been introduced to improve the concentration sensitivity of CE. On-column sample concentration approaches, particularly on-line analyte focusing by cITP, have drawn the most attention [95,100,115,119–125]. In contrast to CE which lacks good concentration sensitivity necessary for most real-world applications while providing very high separation efficiency, cITP offers excellent concentration sensitivity but suffers from the resolution required to characterize complex mixtures. In cITP, the isotachophoretically stacked bands may be several orders of magnitude more concentrated than was originally loaded into the capillary. Extremely narrow zones, frequently less than 1 s wide, overlap one another, a characteristic found in cITP separations with trace quantities of compounds which requires the mass spectrometer to scan at extremely high speed in order to generate useful data. Although spacers may be used to improve the separation, it is generally impractical when a complex mixture of unknown components is involved. The combination of CE and cITP has the potential to overcome the disadvantages of both techniques while retaining their advantages.

Some applications using cITP preconcentration involve two separate capillaries [120,121,124]. In these designs, preconcentration by cITP takes place in one capillary. The isotachophoretically focused sample plugs are then transferred into a second capillary where CE separation takes place. The coupling of cITP and CE is accomplished by inserting the CE capillary into the cITP capillary through a septum. A schematic illustration of the system is show in Fig. 13. A 200-fold improvement in concentration sensitivity for the determination of anthracyclines was achieved by Van der Greef and co-workers [120,124]. The authors have derived an equation for the calculation of the splitting ratio of cITP zones into the CE–ESI-MS system [121].

The coupled use of cITP and CE within the same capillary approach seems to be more effective in terms of ease of application and interfacing to the mass spectrometer [95,100,115,119,122,123,125]. The technique can be applied to any CE–MS system without modification of instrumentation. Using on-line transient cITP preconcentration techniques, the detection limit of some protein samples was improved by two orders of magnitude [95,125]. An example full-

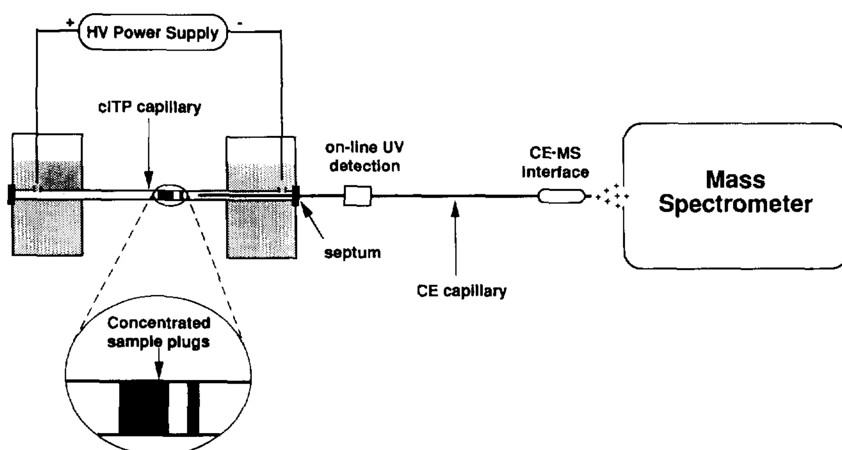

Fig. 13. Schematic illustration of on-line cITP preconcentration for CE–MS using two separate capillaries.

example full-scan CE–ESI-MS electropherogram of a synthetic protein mixture obtained using on-line transient cITP preconcentration is shown in Fig. 14. As much as 750 nl of sample was injected. Full-scan CE–ESI-MS spectra were obtained with analytes at concentrations as low as ca. 10^{-7} *M*, which was 100-fold lower than CE–ESI-MS without preconcentration. A similar approach was described by Lamoree and co-workers [115,123]. A sample volume of 870 nl test mixture containing several β-agonists was analyzed without sacrificing separation resolution. The capability of the cITP–CE–ESI-MS system was demonstrated for the trace analysis of a complex matrix such as calf urine extracts. Recently, several research groups have evaluated the cITP–CE–ESI-MS approach [100,119,122]. On-line cITP preconcentration has lowered the concentration detection limits of CE–MS by two orders of magnitude for the analysis of oligosaccharides [100]. Similar results were obtained by Mosely [122] who observed a 200-fold improvement in concentration limits by using the preconcentration approach, with up to 45% of the CE capillary being initially filled with sample. It was found that the greater the difference in the electrophoretic mobilities between the terminating electrolyte and the analyte, the better the cITP focusing becomes. Analytes with low electrophoretic mobilities were not preconcentrated. In all cases, the on-line cITP preconcentration has reduced the separation efficiency. But they were still much better than those of conventional LC separations.

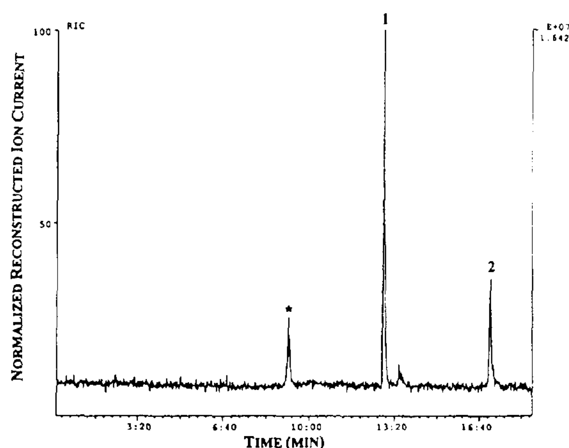

Fig. 14. Transient cITP–MS full-scan reconstructed ion electropherogram of synthetic protein mixture with 750 nl injection. Peak: 1 = cytochrome *c*; 2 = myoglobin; * = from the rear boundary of loading buffer. From Ref. [95].

4.2.2. Chromatographic on-line preconcentration

Another approach for on-line analyte concentration for CE is the chromatographic method. This technique involves a precolumn, either having walls derivatized with C_{18} and other functionalities [126,127] or packed with HPLC packing materials [128,129]. Recently, this concept has been incorporated into CE–ESI-MS

applications [92,93]. The precolumn was made of a small bed of HPLC packing in a PTFE cartridge attached to the inlet of the CE capillary. It serves as an on-line device for sample cleanup such as desalting and preconcentration for subsequent analysis by CE–ESI-MS.

4.2.3. Other analytical approaches

The use of small-I.D. capillaries was found to have improved the absolute sensitivity of CE–ESI-MS [16,17]. This improvement in sensitivity is due to the high ionization efficiency associated with the very low bulk flow-rate from the small-I.D. capillary into the mass spectrometer. Since the ESI ion current is nearly independent of flow-rate, ESI-MS will operate as a mass-sensitive detection system when the ESI current is limited by the flow-rate of the charged species in the solution to the ESI source [34]. This is illustrated in Fig. 15 where a 25- to 50-fold increase in absolute sensitivity is obtained by reducing the I.D. of CE capillary from 100 to 10 μm.

Frequently, the high resolution observed with CE separation results in extremely narrow peaks

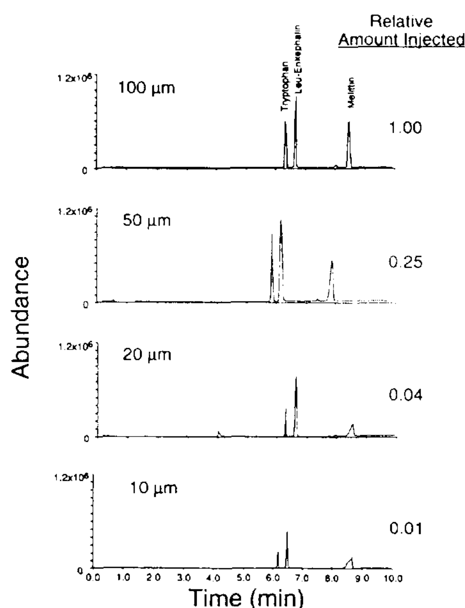

Fig. 15. CE–ESI-SIM-MS total ion current electropherograms of a synthetic peptide mixture using 100, 50, 20, 10 μm I.D. capillaries. From Ref. [17].

and are sometimes not suitable for performing on-line CE–MS, especially full-scan on-line MS analysis. By reducing the applied electric field strength just prior to the elution of the analyte, the peaks were broadened sufficiently to allow enough scans be obtained and averaged across each peak [18,100].

Recently Kirby et al. [130] demonstrated that by carefully choosing the experimental conditions, surfactants can be used successfully in CE–ESI-MS with only minimal loss in analyte sensitivity. They also found that the use of mixed surfactant resulted in much less analyte signal suppression than what is expected by using sodium dodecyl sulfate alone.

4.3. Instrumental developments

4.3.1. Coated capillaries

Coated capillaries are often used in the analysis of biological samples such as basic peptides and proteins to minimize band broadening and peak tailing due to adsorption of analytes onto the capillary walls [131]. The factors that attribute to protein adsorption include ionic, hydrophobic, and hydrogen-bonding interactions [3]. The recent advances in CE capillary coatings have been reviewed [132]. Coated capillaries are currently available from several commercial sources. Aminopropyl silylated fused-silica capillaries have frequently been used in CE–MS applications [17,18,20,21,33,34,36,73,74,89,104, 133]. Capillaries coated with polyacrylamide have been used to minimize protein adsorption and electroosmotic flow within the CE capillary for the analysis of proteins by CE–MS [30,31,95,130]. A different hydrophilic derivatized capillary was prepared for protein analysis by CE–MS [94]. Kostiainen et al. [54] used a commercially available deactivated fused-silica capillary for the determination of a synthetic drug–protein conjugate mixture by CE–MS. Thibault et al. [47] described an CE–MS application using a non-covalent coated CE capillary with an overall positive charge. The use of coated capillaries to eliminate the electroosmotic flow may also be useful in minimizing the flow of non-volatile additives into the mass spectrometer

[130]. This may be of particular importance in applications such as chiral separations by CE–MS.

4.3.2. Trapped ion mass spectrometers

One solution to improving the sensitivity of CE–MS is the development of alternative types of mass spectrometers which offer the potential for greater sensitivity. Currently the efficiency of ion transport from the atmospheric pressure ESI source to the MS detector is low with only approximately 10^{-5} of the ions formed in ESI being detected [134]. Trapped ion mass spectrometers, include the quadrupole ion trap (IT) and the FTICR mass spectrometer may be useful for improving CE–MS sensitivity. IT mass spectrometers provide significantly higher transmission efficiencies than beam-type mass spectrometers [135]. The coupling of CE with IT-MS has been demonstrated in several research groups [36,49,90,136,137]. Ramsey et al. [136] reported chemical background and noise reduction in CE–MS on a quadrupole IT mass spectrometer. By using the combination of broad-band collisional activation and resonance ejection, they were able to significantly reduce background noise in the CE–ESI-MS total ion electropherograms. The electropherograms obtained before and after the background reduction are shown in Fig. 16. Recently we have demonstrated the coupling of CE to a highly modified benchtop IT mass spectrometer for quantitative determination some Chinese herbal remedies [49]. A signal-to-noise ratio better than 10 was obtained in the extracted ion current with injected quantities between 370 to 510 attomole (see Fig. 17).

FTICR-MS has the advantages of high sensitivity, ultra-high resolution and extensive capabilities for tandem MS (MS^n) [138,139]. The combination of CE and FTICR-MS has been reported [97,140,141]. An off-line coupling of CE and MALDI-MS was demonstrated on a FTICR mass spectrometer [142]. Hofstadler et al. [140] have modified a FTICR instrument which allows rapid cycling between the different pressure regimes in the trapped ion cell for both optimum trapping efficiency and high-resolution detection. The CE–ESI-MS experiments on the

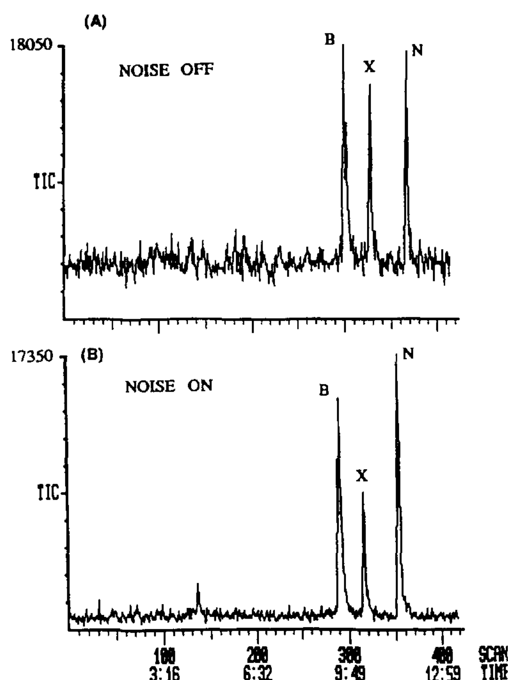

Fig. 16. Total ion electropherograms (TIC) of a synthetic peptide mixture obtained (A) without active background reduction measures and (B) with combined broad-band collisional activation and resonance ejection. Peaks: B = bradykinin; X = xenopsin; N = neurotensin. Time in min. From Ref. [136].

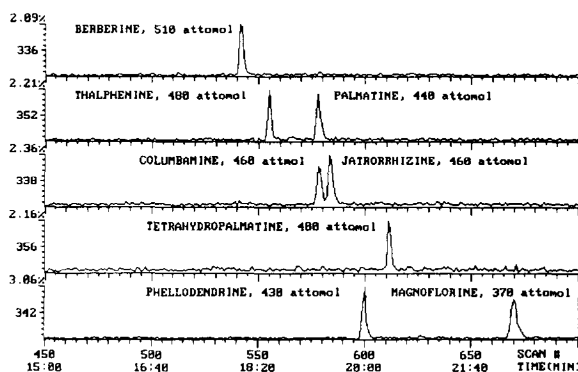

Fig. 17. Full-scan CE–ISP-MS extracted ion current electropherograms of a synthetic mixture containing isoquinoline alkaloid natural products at low levels obtained on a modified benchtop ion trap mass spectrometer. Low "up-front" collision induced dissociation (CID) = 52.4 V; 25 μm I.D. capillary; mass range 150–400; 100% = 27 408. From Ref. [49].

FTICR instrumentation allows fast spectrum acquisition speed. The potential of the CE–ESI-FTICR-MS system was further evaluated for a chymotryptic digest of ubiquitin [97]. The on-line combination of CE with ESI-FTICR-MS has also been presented by Johnson et al. [141]. A pulsed collision gas of nitrogen was used to collisionally trap ions. Trapping efficiency of ca. 1% was reported. However, due to the limited pumping speed, high-resolution mass spectra could not be obtained immediately after ion accumulation. The ions were stored in the FTICR cell for 10 min before a reasonably low base pressure could be reached for high-resolution detection.

4.3.3. *Time of flight*

TOF mass spectrometers offer very rapid scan rates with the ability to record a full mass spectrum from an injection pulse [14]. This makes it potentially good for on-line applications such as CE–MS and LC–MS. In addition, TOF analyzers can provide improved resolution by using a reflectron configuration. Also, they are relatively simple and inexpensive, and are well suited for pulsed ionization techniques such as MALDI. The use of TOF MS on-line with CE has been published by Zare and co-workers [35,143]. The applicability of the system was demonstrated using a sheathless ESI interface for the characterization of synthetic peptide mixtures. Recently, the same research group has reported a modified ion optics system which greatly improved ion transmission efficiencies [144]. Detection limits at tens of femtomole injected on-column were obtained for synthetic peptides. The off-line combination of CE and MALDI-TOF-MS has also been reported using fraction collection [86].

4.3.4. *Use of an array detector*

The use of a position and time-resolved ion counting (PATRIC) detector was found to have dramatically improved the sensitivity of mass spectral detection in CE–MS [145]. Such PATRIC array detectors are available on Finnigan MAT Model 900 mass spectrometers. While the static detection mode was found to be useful in target compound analysis, scanning array detection was more universal. By using scanning array detection, a significant gain in sensitivity (10- to 100-fold) can be obtained over a conventional secondary electron multiplier detector in combination with scanning. A same instrument equipped with PATRIC has been used in CE–ESI-MS applications of drug metabolism studies [22,24,113,114].

Acknowledgements

We acknowledge Beckman Instruments for the loan of the P/ACE 2050 CE instrument, and Eastman Kodak and PE Sciex for partial support of our CE–MS research.

References

[1] J. Jorgenson and K. Lukacs, *Anal. Chem.*, 53 (1981) 1298–1302.
[2] R. Wallingford and A. Ewing, *Anal. Chem.*, 60 (1988) 1972–1975.
[3] C.A. Monning and R.D. Kennedy, *Anal. Chem.*, 66 (1994) 280R–314R.
[4] J.A. Olivares, N.T. Nguyen, C.R. Yonker and R.D. Smith, *Anal. Chem.*, 59 (1987) 1230–1232.
[5] R.D. Smith, J.H. Wahl and D.R. Goodlett, *Anal. Chem.*, 65 (1993) 574A–584A.
[6] W.M.A. Niessen, U.R. Tjaden and J. van der Greef, *J. Chromatogr.*, 636 (1993) 3–19.
[7] J. van der Greef and W.M.A. Niessen, *Int. J. Mass Spectrom. Ion Processes*, 118/119 (1992) 857–873.
[8] P. Kebarle and L. Tang, *Anal. Chem.*, 65 (1993) 972A–986A.
[9] J.V. Iribarne and B.A. Thomson, *J. Chem. Phys.*, 64 (1976) 2287–2282.
[10] G. Schmelzeisen-Redeker, L. Buttering and F.W. Rollgen, *Int. J. Mass Spectrom. Ion Processes*, 90 (1989) 139–150.
[11] J. Zeleny, *Phys. Rev.*, 10 (1917) 1–6.
[12] M. Yamashita and J.B. Fenn, *J. Phys. Chem.*, 88 (1984) 4451–4459.
[13] C.M. Whitehouse, R.N. Dreyer, M. Yamashita and J.B. Fenn, *Anal. Chem.*, 57 (1985) 675–679.
[14] A.L. Burlingame, R.K. Boyd and S.J. Gaskell, *Anal. Chem.*, 66 (1994) 634R–683R.
[15] R.D. Smith, C.J. Barinaga and H.R. Udseth, *Anal. Chem.*, 60 (1988) 1948–1952.
[16] J.H. Wahl, D.R. Goodlett, H.R. Udseth and R.D. Smith, *Anal. Chem.*, 64 (1992) 3194–3196.

[17] J.H. Wahl, D.R. Goodlett and H.R. Udseth, *Electrophoresis*, 14 (1993) 448–457.
[18] D.R. Goodlett, J.H. Wahl, H.R. Udseth and R.D. Smith, *J. Microcol. Sep.*, 5 (1993) 57–62.
[19] K. Tsuji, L. Baczynskj and G.E. Bronson, *Anal. Chem.*, 64 (1992) 1864–1870.
[20] M.A. Moseley, J.W. Jorgenson, J. Shabanowitz, D.F. Hunt and K.B. Tomer, *J. Am. Soc. Mass Spectrom.*, 3 (1992) 289–300.
[21] J.R. Perkins, C.E. Parker and K.B. Tomer, *Electrophoresis*, 14 (1993) 458–468.
[22] A.J. Tomlinson, L.M. Benson and S. Naylor, *Am. Lab.*, June (1994) 29–36.
[23] A.J. Tomlinson, L.M. Benson and S. Naylor, *LC·GC*, 12 (1994) 122–130.
[24] A.J. Tomlinson, L.M. Benson, K.L. Johnson and S. Naylor, *J. Chromatogr.*, 621 (1993) 239–248.
[25] J. Varghese and R.B. Cole, *J. Chromatogr.*, 639 (1993) 303–316.
[26] J.V. Varghese and R.B. Cole, *J. Chromatogr. A*, 652 (1993) 369–372.
[27] J.R. Perkins, C.E. Parker and K.B. Tomer, *J. Am. Soc. Mass Spectrom.*, 3 (1992) 139–149.
[28] C.E. Parker, J.R. Perkins, K.B. Tomer, Y. Shida, K. O'Hara and M. Kono, *J. Am. Soc. Mass Spectrom.*, 3 (1992) 563–574.
[29] D.C. Gale and R.D. Smith, *Rapid Commun. Mass Spectrom.*, 7 (1993) 1017–1021.
[30] T.J. Thompson, F. Foret, D.P. Kirby, P. Vouros and B.L. Karger, presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 31 May–4 June 1993.*
[31] T.J. Thompson, F. Foret, B.L. Karger and P. Vouros, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*
[32] S.K. Chowdhury and B.T. Chait, *Anal. Chem.*, 63 (1991) 1660–1664.
[33] J.H. Wahl and R.D. Smith, *J. Cap. Elec.*, 1 (1994) 62–71.
[34] J.H. Wahl, D.C. Gale and R.D. Smith, *J. Chromatogr. A*, 659 (1994) 217–222.
[35] L. Fang, R. Zhang and R.N. Zare, presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 31 May–4 June 1993.*
[36] M.S. Kriger, R.S. Ramsey and K.D. Cook, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*
[37] G. Hopfgartner, T. Wachs, K. Bean and J. Henion, *Anal. Chem.*, 65 (1993) 439–446.
[38] M.G. Ikonomou, A.T. Blades and P. Kebarle, *Anal. Chem.*, 63 (1991) 1989–1998.
[39] E.D. Lee, W. Muck, J.D. Henion and T.R. Covey, *Biomed. Environ. Mass Spectrom.*, 18 (1989) 844–850.
[40] E.D. Lee, W. Muck, J.D. Henion and T.R. Covey, *J. Chromatogr.*, 458 (1988) 313–321.
[41] J.M. Johansson, E.C. Huang, J.D. Henion and J. Zweigenbaum, *J. Chromatogr.*, 554 (1991) 311–327.
[42] F. Garcia and J. Henion, *J. Chromatogr.*, 606 (1992) 237–47.
[43] E.D. Lee, W. Muck, J.D. Henion and T.R. Covey, *Biomed. Environ. Mass Spectrom.*, 18 (1989) 253–257.
[44] J.M. Johansson, R. Pavelka and J.D. Henion, *J. Chromatogr.*, 559 (1991) 515–528.
[45] F. Garcia and J.D. Henion, *Anal. Chem.*, 64 (1992) 985–990.
[46] P. Thibault, S. Pleasance and M.V. Laycock, *J. Chromatogr.*, 542 (1991) 483–501.
[47] P. Thibault, C. Paris and S. Pleasance, *Rapid Commun. Mass Spectrom.*, 5 (1991) 484–490.
[48] S. Pleasance, P. Thibault and J. Kelly, *J. Chromatogr.*, 591 (1992) 325–339.
[49] J. Henion, A. Mordehai and J. Cai, *Anal. Chem.*, 66 (1994) 2103–2109.
[50] L.W. Tetler, P.A. Cooper and B. Powell, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*
[51] D. Wycherley, M.E. Rose, K. Giles, S.A. Jarvis and M.A. McDowall, presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 31 May–4 June 1993.*
[52] K.J. Volk, J. Liu, M.S. Lee, S.E. Klohr, E.H. Kerns and I.E. Rosenberg, presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 31 May–4 June 1993.*
[53] R. Kostiainen, E. Lasonder and W. Bloemhoff, *Biol. Mass Spectrom.*, 23 (1994) 346–352.
[54] R. Kostiainen, E.J.F. Franssen and A.P. Bruins, *J. Chromatogr.*, 647 (1993) 361–365.
[55] J. Cai, H.K. Lim and J. Henion, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*
[56] T.G. Huggins and J.D. Henion, *Electrophoresis*, 14 (1993) 531–539.
[57] J.J. Corr, T.R. Covey and J.F. Anacleto, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*
[58] L.W. Tetler, P.A. Cooper and C.M. Carr, *Rapid Commun. Mass Spectrom.*, 8 (1994) 179–182.
[59] R. Kostiainen, A.P. Bruins and V.M.A. Hakkinen, *J. Chromatogr.*, 634 (1993) 113–118.
[60] S. Pleasance, S.W. Ayer, M.V. Laycock and P. Thibault, *Rapid Commun. Mass Spectrom.*, 6 (1992) 14–24.
[61] D. Wycherley, M.E. Rose, D. Rimmer, K. Giles, S.A. Jarvis and M.A. McDowall, presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 31 May–4 June 1993.*
[62] M. Barber, R.S. Bordoli, R.D. Sedgwick and A. Tyler N., *J. Chem. Soc., Chem. Commun.*, (1981) 325–327.
[63] Y. Ito, T. Takeuchi, D. Ishi and M. Goto, *J. Chromatogr.*, 346 (1985) 161–166.
[64] R.D. Minard, D. Chin-Fatt, P.J. Carry and A.G.

Ewing, presented at the *36th ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 5–10 June 1988.*

[65] N.J. Reinhoud, W.M.A. Niessen, U.R. Tjaden, L.G. Gramberg, E.R. Verheij and J. van der Greef, *Rapid Commun. Mass Spectrom.*, 3 (1989) 348–351.

[66] R.D. Minard, D. Luckenbill, P.J. Curry and A.G. Ewing, *Adv. Mass Spectrom.*, 11 (1989) 436–437.

[67] R.M. Caprioli, W.T. Moore, M. Martin, B.B. DaGue, K. Wilson and S. Moring, *J. Chromatogr.*, 480 (1989) 247–257.

[68] S.M. Wolf, P. Vouros, C. Norwood and E. Jakim, *J. Am. Soc. Mass Spectrom.*, 3 (1992) 757–761.

[69] J.S.M. de Wit, L.J. Deterding, M.A. Moseley, K.B. Tomer and J.W. Jorgenson, *Rapid Commun. Mass Spectrom.*, 2 (1988) 100–104.

[70] M.A. Moseley, L.J. Deterding, K.B. Tomer and J.W. Jorgenson, *Rapid Commun. Mass Spectrom.*, 3 (1989) 87–93.

[71] M.A. Moseley, L.J. Deterding, K.B. Tomer and J.W. Jorgenson, *J. Chromatogr.*, 480 (1989) 197–209.

[72] M.A. Moseley, L.J. Deterding, K.B. Tomer and J.W. Jorgenson, *J. Chromatogr.*, 516 (1990) 167–173.

[73] M.A. Moseley, L.J. Deterding, K.B. Tomer and J.W. Jorgenson, *Anal. Chem.*, 63 (1991) 109–114.

[74] L.J. Deterding, C.E. Parker, J.R. Perkins, M.A. Moseley, J.W. Jorgenson and K.B. Tomer, *J. Chromatogr.*, 554 (1991) 329–338.

[75] K.B. Tomer, L.J. Deterding, S.R. McGown and J.R. Perkins, presented at the *40th ASMS Conference on Mass Spectrometry and Allied Topics, Washington, DC, 31 May–5 June 1992.*

[76] M.J.-F. Suter and R.M. Caprioli, *J. Am. Soc. Mass Spectrom.*, 3 (1992) 198–206.

[77] W. Nichols, J. Zweigenbaum, F. Garcia, M. Johansson and J. Henion, *LC·GC*, 10 (1992) 676–686.

[78] C.G. Edmonds, J.A. Loo and R.R. Ogorzalek Loo, *Biochem. Soc. Trans.*, 19 (1991) 943–947.

[79] M.A. Tarr, G. Zhu and R.F. Browner, *Anal. Chem.*, 65 (1993) 1689–1695.

[80] M. Karas, U. Bahr, A. Ingendoh, E. Nordhoff, B. Stahl, K. Stupat and F. Hillenkamp, *Anal. Chim. Acta*, 241 (1990) 175–185.

[81] R. Takigiku, T. Keough, M.P. Lacey and R.E. Schneider, *Rapid Commun. Mass Spectrom.*, 4 (1990) 24–29.

[82] T. Keough, R. Takigiku, M.P. Lacey and M. Purdon, *Anal. Chem.*, 64 (1992) 1594–1600.

[83] W. Weinmann, K. Baumeister, I. Kaufmann and M. Przybylski, *J. Chromatogr.*, 628 (1993) 111–121.

[84] W. Weinmann, C.E. Parker, K. Baumeister, C. Maier, K.B. Tomer and M. Przybylski, *Electrophoresis*, 15 (1994) 228–233.

[85] P.A. van Veelen, U.R. Tjaden and J. van der Greef, *J. Chromatogr.*, 647 (1993) 367–374.

[86] K.B. Tomer, W. Weinmann, D. Papac, L. Palm, C.E. Parker, L. Deterding, M. Przybylski and J. Hoyes, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[87] C.R. Blakley, M.L. Vestal and A. Verentchikov, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[88] A.F. Lecoq, S. Di Biase and L. Montanarella, *J. Chromatogr.*, 638 (1993) 363–373.

[89] D.F. Hunt, J.E. Alexander, A.L. McCormack, P.A. Martino, H. Michel, J. Shabanowitz and N. Sherman, in J.J. Villafranca (Editor), *Techniques in Protein Chemistry II*, Academic Press, New York, 1991, pp. 441–454.

[90] R.S. Ramsey, K.G. Asano, K.J. Hart, D.E. Goeringer, G.J. van Berkel and S.A. Mcluckey, presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 31 May–4 June 1993.*

[91] K.J. Rosnack, J.G. Stroh, G.C. Adrews and D.H. Singleton, presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 31 May–4 June 1993.*

[92] A.J. Tomlinson, W.K. Nevala, W.D. Braddock, M.A. Strausbauch, P.J. Wettstein and S. Naylor, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[93] A.J. Tomlinson, L.M. Benson, M.A. Strausbauch, G.F. Scanlan, P.J. Wettstein, F. Rusnak and S. Naylor, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[94] R.B. Cole, J. Varghese and R.M. McCormick, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[95] T.J. Thompson, F. Foret, P. Vouros and B.L. Karger, *Anal. Chem.*, 65 (1993) 900–906.

[96] R.D. Smith, H.R. Udseth, C.J. Barinaga and C.G. Edmonds, *J. Chromatogr.*, 559 (1991) 197–208.

[97] J.H. Wahl, S.A. Hofstadler, Z. Zhao, D.C. Gale and R.D. Smith, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[98] R. Takigiku, T. Keough, M.P. Lacey, M.P. Purdon, L. Licklider and W.G. Kuhr, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[99] J.F. Kelly, P. Thibault, H. Masoud, M.B. Perry and J.C. Richards, presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 31 May–4 June 1993.*

[100] J.F. Kelly, P. Thibault, S. Locke and L.R. Ramaley,

presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[101] Z. Zhao, J.H. Wahl, A.F. Fuciarelli, D.C. Gale, H.R. Udseth and R.D. Smith, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[102] J.P. Barry, P. Vouros and C.B. Norwood, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[103] F.Y.-L. Hsieh, J. Cai, Y.-T. Li, B. Ganem and J. Henion, *J. Am. Soc. Mass Spectrom.*, in press.

[104] D.R. Goodlett, R.R.O. Loo, J.A. Loo, J.H. Wahl, H.R. Udseth and R.D. Smith, *J. Am. Soc. Mass Spectrom.*, 5 (1994) 614–622.

[105] F. Uzabiaga, A. Tuong, J.C. Lormeau, M. Petitou and C. Picard, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[106] I. Mylchreest, presented at the *40th ASMS Conference on Mass Spectrometry and Allied Topics, Washington, DC, 31 May–5 June 1992.*

[107] J. Cai, A. Mordehai and J. Henion, presented at the *10th Symposium on Liquid Chromatography/Mass Spectrometry, Ithaca, NY, 21–23 July 1993.*

[108] F.Y.-L. Hsieh, J. Cai and J. Henion, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[109] S.C. Stinson, *Chem. Eng. News*, (1994) 38–72.

[110] T.J. Ward, *Anal. Chem.*, 66 (1994) 633A–640A.

[111] M. Novotny, H. Soini and M. Stefansson, *Anal. Chem.*, 66 (1994) 646A–655A.

[112] R. Sheppard, X. Tong, J. Cai and J. Henion, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[113] A.J. Tomlinson, L.M. Benson and S. Naylor, *Electrophoresis*, 15 (1994) 62–71.

[114] A.J. Tomlinson, L.M. Benson and S. Naylor, *J. High Resolut. Chromatogr.*, 17 (1994) 175–177.

[115] M.H. Lamoree, N.J. Reinhoud and U.R. Tjaden, *Biol. Mass Spectrom.*, 23 (1994) 339–345.

[116] H.B. Hines, M. Holcomb and E. Brueggemann, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[117] M.D. LeBlanc and L. Ramaley, presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 31 May–4 June 1993.*

[118] W.M. Muck and J.D. Henion, *J. Chromatogr.*, 495 (1989) 41–59.

[119] J.C. Severs and D.E. Games, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[120] A.P. Tinke, N.J. Reinhoud, W.M.A. Niessen, U.R. Tjaden and J. van der Greef, *Rapid Commun. Mass Spectrom.*, 6 (1992) 560–563.

[121] N.J. Reinhoud, A.P. Tinke, U.R. Tjaden, W.M.A. Niessen and J. van der Greef, *J. Chromatogr.*, 627 (1992) 263–271.

[122] M.A. Moseley, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[123] M.H. Lamoree, N.J. Reinhoud, U.R. Tjaden, W.M.A. Niessen and J. Van der Greef, presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 31 May–4 June 1993.*

[124] J. Van der Greef, A.P. Tinke, N.J. Reinhoud, W.M.A. Niessen and U.R. Tjaden, presented at the *40th ASMS Conference on Mass Spectrometry and Allied Topics, Washington, DC, 31 May–5 June 1992.*

[125] T.J. Thompson, P. Vouros, F. Foret and B. Karger, presented at the *40th ASMS Conference on Mass Spectrometry and Allied Topics, Washington, DC, 31 May–5 June 1992.*

[126] J. Cai and Z. El Rassi, *J. Liq. Chromatogr.*, 15 (1992) 2007–2024.

[127] J. Cai and Z. El Rassi, *J. Liq. Chromatogr.*, 15 (1992) 1179–1192.

[128] I. Morita and J.I. Sawada, *J. Chromatogr.*, 641 (1993) 375–381.

[129] M.E. Swartz and M. Merion, *J. Chromatogr.*, 632 (1993) 209–213.

[130] D. Kirby, K.F. Greve, F. Foret, P. Vouros, B.L. Karger and W. Nashabeh, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[131] J. Kohr and H. Engelhardt, *J. Chromatogr.*, 652 (1993) 309–316.

[132] T. Wehr, *LC·GC*, 11 (1993) 14–20.

[133] W. Weinmann, M. Przybylski, C.E. Parker and K.B. Tomer, presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 31 May–4 June 1993.*

[134] R.D. Smith, J.A. Loo, C.G. Edmonds, C.J. Barinaga and H.R. Udseth, *J. Chromatogr.*, 516 (1990) 157–165.

[135] S.A. McLuckey, G.J. van Berkel, D.E. Goeringer and G.L. Glish, *Anal. Chem.*, 66 (1994) 689A–696A.

[136] R.S. Ramsey, D.E. Goeringer and S.A. Mcluckey, *Anal. Chem.*, 65 (1993) 3521–3524.

[137] J.C. Schwartz and I. Jardine, presented at the *40th ASMS Conference on Mass Spectrometry and Allied Topics, Washington, DC, 31 May–5 June 1992.*

[138] K.D. Henry, J.P. Quinn and F.W. McLafferty, *J. Am. Chem. Soc.*, 113 (1991) 5447–5450.

[139] M.V. Gorshkov, E.N. Nikolaev, S. Guan, K. Vermillion and A.G. Marshall, presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 31 May–4 June 1993.*

[140] S.A. Hofstadler, J.H. Wahl, J.E. Bruce and R.D. Smith, *J. Am. Chem. Soc.*, 115 (1993) 6983–6984.

[141] P.J. Johnson, P.D. Mills, D.S. Gross, P.D. Schnier and E.R. Williams, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[142] J.A. Castoro, R.W. Chiu, C.A. Monning and C.L. Wilkins, *J. Am. Chem. Soc.*, 114 (1992) 7571–7572.

[143] L. Fang, R. Zhang and R.N. Zare, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[144] R. Zhang, L. Fang and R.N. Zare, presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL, 29 May–3 June 1994.*

[145] N.J. Reinhoud, E. Schroder, U.R. Tjaden, W.M.A. Niessen, M.C. ten Noever de Brauw and J. van de Greef, *J. Chromatogr.*, 516 (1990) 147–155.