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Review

Capillary electrophoresis-mass spectrometry

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ABSTRACT

The developments and state-of-the-art in capillary electrophoresis-mass spectrometry (CE-MS) are reviewed and evaluated. Attention is paid to interfaces for CE-MS, the coupling of CE to the interface, applications of CE-MS in both qualitative and quantitative analysis, CE buffer composition and the coupling of other electromigration techniques to MS. The state-of-the-art is critically reviewed. Special attention is paid to the achievable concentration detection limits, because the present limits in the low-ppm range prohibit the broad analytical use of the CE-MS technique. Current approaches to solving or removing these problems are discussed.

CONTENTS

1. INTRODUCTION

Electrophoresis is the migration of electrically charged particles or ions in solution due to an applied electric field. It is the basic principle of a variety of analytical and preparative techniques that are widely applied, especially amongst biochemists [1,2]. In the early 1980s, Jorgenson and Lukacs [3,4] demonstrated that highly efficient separations are possible by the use of high voltage. These high voltages can be applied if the heat dissipation is enhanced by means of a reduction of the internal diameter of the separation tube, e.g., down to 50-100 μm I.D. High-voltage or high-performance capillary zone electrophoresis (denoted CE throughout this text) is an important breakthrough in the analytical application of electrophoresis. Impressive results with CE showing separations with plate numbers in excess of 10^6 have been demonstrated.

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The technique has recently been reviewed in a monograph by Li [5] and by others [6,7].

This paper deals with the coupling of high-voltage capillary zone electrophoresis and mass spectrometry (CE-MS). The on-line combination of high-efficiency separations in capillary electrophoresis and the detection and identification potential of a mass spectrometer is an attractive perspective. CE–MS coupling was investigated soon after the introduction of high-voltage capillary electrophoresis. The first paper on CE-MS was published in 1987 by the Smith's group [8]. This paper reviews the state of the research and critically evaluates the possibilities and limitations of CE-MS.

CE is a liquid-phase separation technique. In order to couple CE with a mass spectrometer, an interface must be present. Most interfaces applied in CE-MS are adaptations of interfaces developed for LC-MS. In practice, a CE-MS system consists of a CE apparatus, a special coupling device, an LC-MS interface and a mass spectrometer.

Typical flow-rates from a CE capillary range from 0 to 100 nl/min, while the current low flow-rate LC-MS interfaces operate in the range of 1–50 μl/min. A make-up liquid is used to solve this flow-rate incompatibility problem.

In setting up the complete system, it is found that the electrical connection at the interface side of the separation capillary is sometimes difficult to accomplish. Unlike in a normal CE set-up, the cathode end of the capillary cannot simply be placed in a buffer vial but must be connected to the CE-MS interface.

Another aspect of CE-MS interfacing is related to the solvent composition of the CE buffer solution. Although the flow-rate of CE buffer is very low, the presence of non-volatile buffer constituents can be detrimental to the (long-term) performance of the MS detection, owing to interface and ion source contamination.

The very high efficiencies that can be achieved in CE can give problems with the scanning speeds of MS. Peaks with migration times of 100 and 1000 s and a plate number of 10^6 have peak standard deviations of 0.1 and 1 s, respectively. At least 6-10 data points, e.g., scans, are necessary for an adequate characterization of the peak. Full scan acquisition over a large mass range can be difficult because the cycle time becomes too short. This is especially true for sector instruments.

Further, with the mass spectrometer principally being a mass flow-sensitive detector, problems might be experienced with the achievable minimum detectable concentrations.

In addition to CE, other electromigration techniques have been coupled to MS. Further, coupling to special MS devices and off-line coupling to plasma and laser desorption time-of-flight instruments have been described.

These topics are discussed in more detail below.

2. INTERFACES FOR CAPILLARY ELECTROPHORESIS–MASS SPECTROMETRY

Three adapted LC-MS interfaces are currently in use for CE-MS: continuous-flow fast atom bombardment (CF-FAB), electrospray and ionspray. General aspects of LC-MS coupling and interface strategies are dealt with in detail elsewhere [9,10]. A concise description of the interfaces used and the working principles is given here.

In a CF-FAB interface, a continuous flow of liquid, typically 5-l 5 μl/min, is introduced through a 50-75 μm I.D. fused-silica capillary to a metal target, which is positioned in a high-vacuum ion source. As in most instances magnetic sector instruments are used for CF-FAB, the ion source is at high potential (4-8 kV). A schematic diagram of the interface is shown in Fig. la. The solvent should contain 1–5% of glycerol to retard evaporation and to serve as matrix in the FAB ionization. The liquid forms a uniform film on the target, from which ionization of the analytes is achieved by bombardment with 8-keV xenon atoms. Part of the liquid evaporates from the target surface, while the excess liquid is collected on to a so-called wick which is a piece of compressed paper placed at the bottom of the ion source. Special features of the CF-FAB interface are discussed in considerably more detail elsewhere [9,11]. The coupling of CE and MS via a CF-FAB interface was first described by Minard *et al.* [12].

In an electrospray interface, a continuous flow of liquid, typically l-10 μl/min, is nebulized as very small droplets (< 1 μm droplet size) into an atmospheric-pressure ion source by the action of a strong electric field on the liquid in a narrow-bore capillary. The evaporation of the solvent from the droplets is assisted by a countercurrent heated nitrogen flow.

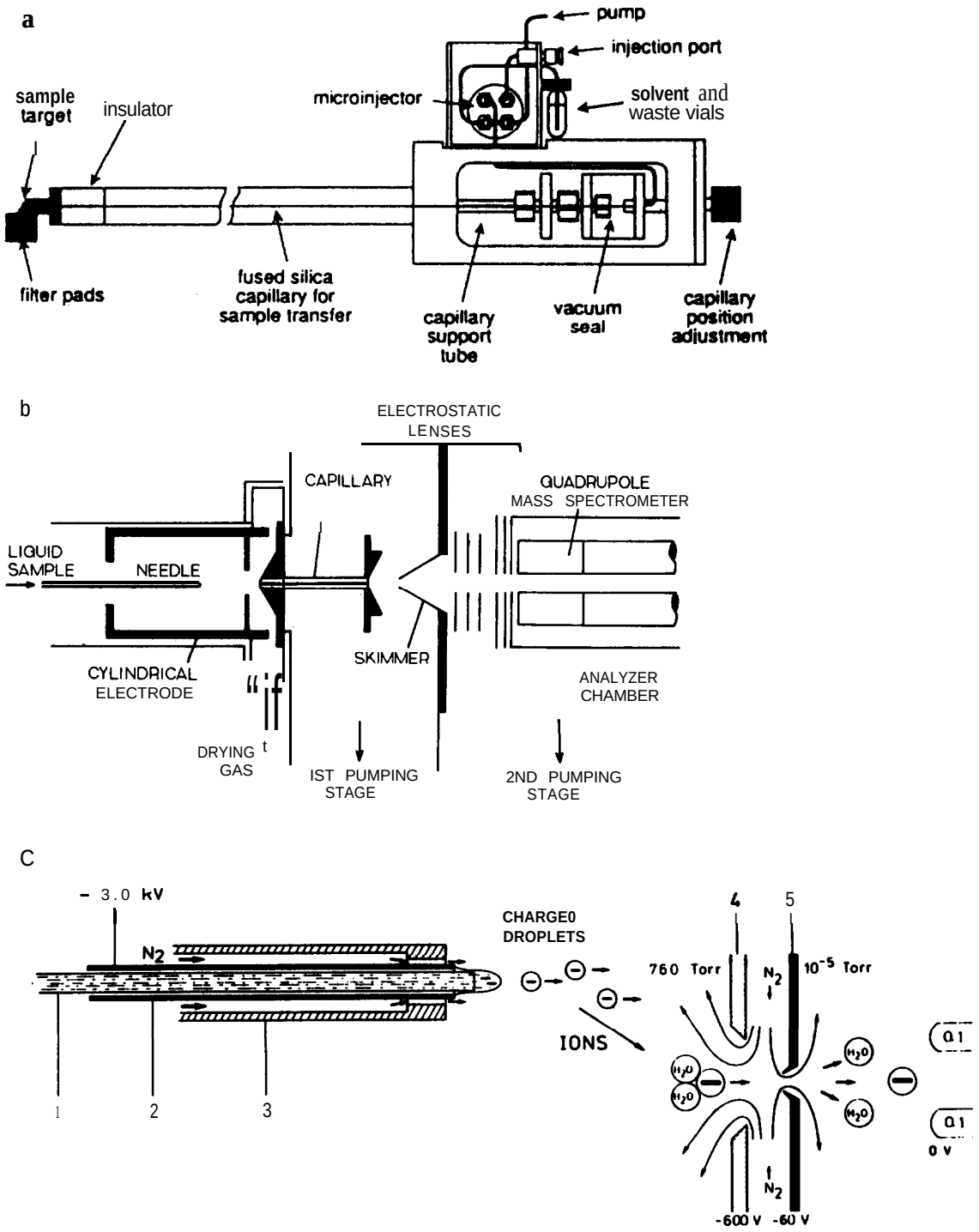

Fig. 1. Schematic diagrams of LC-MS interfaces used in CE-MS coupling. (a) Continuous-flow fast atom bombardment interface; (b) electrospray interface; (c) ionspray interface. From (a) ref. 11 © 1990, Wiley), (b) ref. 13 (© 1985, American Chemical Society) and (c) ref. 14 (© 1987, American Chemical Society). I Torr = 133.322 Pa.

Analytes are ionized from the droplets by the ion evaporation process or as a result of gas-phase ion–molecule reactions. The ions are introduced through a small pinhole or a narrow-bore capillary into the high vacuum of the mass spectrometer. A schematic diagram of the electrospray interface, as originally designed by Whitehouse *et al.* [13], is shown in Fig. lb. In this type of electrospray interface the spray needle is at ground potential, while the counter electrode is at a potential of 3-5 kV. Other electrospray systems have been described in which the needle is at 3-5 kV relative to a grounded counter electrode. This difference has some consequences for the experimental set-up. CE-MS coupling using an electrospray interface was first described by Smith's group [8].

The ionspray interface [14] is also an interface based on an atmospheric-pressure ion source. The major difference from electrospray is that in the ionspray interface the nebulization is assisted by a gas stream surrounding the electrospray needle. As a result, higher flow-rates, typically up to 50 μl/min, can be introduced. A schematic diagram of the ionspray interface is shown in Fig. lc. From Fig. 1 b and c it can be concluded that there are basically two designs for the transition region between atmospheric pressure and the high vacuum of the mass spectrometer, i.e., via a glass capillary and a differentially pumped nozzle-skimmer arrangement [13] or via a pinhole and the use of high-efficiency cryopumps [14]. These aspects are discussed in more detail by Bruins [15]. CE-MS coupling via an ionspray interface was first described by Henion's group [16].

Some other approaches to CE-MS coupling are discussed in a separate section below.

3. COUPLING OF CAPILLARY ELECTROPHORESIS TO THE INTERFACE

Typical flow-rates from the CE separation capillary are in the range 0–100 nl/min. Currently used LC-MS interfaces cannot operate at such low flow-rates; at least 5-10 μl/min are required on CF-FAB and 0.2-l μl/min in electrospray. The problems in coupling CE and MS are thus identical with those met in the on-line coupling of open-tubular LC and MS [17]. The general solution to this problem is the postcapillary addition of a make-up liquid which serves not only as an additional flow but also in establishing electrical contact between the CE buffer and the electrode. The make-up liquid can be added by means of a coaxial capillary, as first demonstrated by Niessen and Poppe [18] for open-tubular LC-MS using a direct-liquid introduction interface and subsequently adapted for continuous-flow fast atom bombardment by De Wit *et al.* [19]. Obviously, the make-up liquid can also be added by means of a low-dead-volume T-piece between the separation capillary and the CE-MS interface. The specially designed T-piece used is called a liquid-junction coupling. Extreme care must be taken not to lose the separation efficiency and resolution of CE owing to external peak broadening in these coupling devices, especially in the latter.

The first experiments in CE-MS were described by Smith's group [8] in 1987. The interface was based on the electrospray LC-MS interface described by Whitehouse *et al.* [13]. A major difference between these two electrospray interfaces is that in the Whitehouse interface the needle is at ground potential relative to a cylindrical counter electrode at 3-5 kV, whereas in the Smith interface the needle is at a potential of 3-5 kV relative to a grounded plate. Therefore, in the latter design the low-voltage end of the CE capillary cannot be at ground potential, as in a conventional CE set-up, but must be at a potential of a few kilovolts. In the first prototype [8,20], the low-voltage end of the 100 μm I.D. fused-silica CE separation capillary was surrounded by a 300 μm I.D. x 450 μm O.D. stainless-steel capillary, kept at O-5 kV and functioning both as the CE cathode and as the electrospray needle. Relatively large capillary diameters were required to achieve a sufficiently high flow-rate to sustain stable electrospray performance. In the second prototype [21], the electric contact to the buffer solution was improved by the deposition of silver on the fused-silica capillary and the stainless-steel sheath. A further improved interface was subsequently developed in which a liquid sheath flow surrounding the CE capillary provided for both the electric contact at the cathode end of the CE capillary and the suitable solvent conditions for the electrospray, independent of the CE buffer solution [22,23]. A schematic diagram of the interface is shown in Fig. 2. This set-up also permitted the use of separation capillaries of smaller I.D., *i.e.*, $\leqslant 75$ μm.

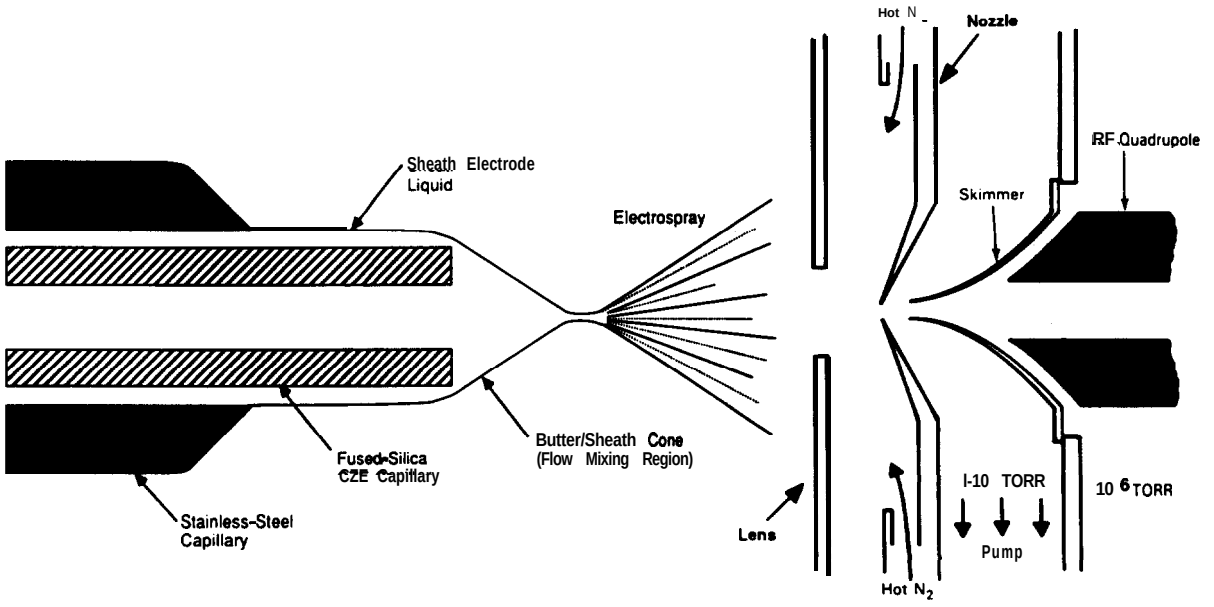

Fig. 2. Schematic diagram of the electrospray interface for CE-MS as described by Smith and co-workers [22,23]. From ref. 23 (© 1989, Academic Press).

The improved interface of Smith and co-workers [22,23] is an example of a coaxial delivery of make-up liquid. Coaxial coupling for CE-MS have been described not only for electrospray, but also for CF-FAB and ionspray.

Coaxial make-up liquid addition for CE-MS via CF-FAB was first described by Moseley *et al.* [24,25] based on a design for LC-MS coupling [19]. This is an elegant approach, as it allows for independent optimization of the flow-rate and composition of the liquids in CE separation capillary and make-up liquid. Further, it permits the addition of the glycerol necessary as a FAB matrix without significant peak broadening. However, as the outlet end of the CE capillary is in the high-vacuum ion source, a significant vacuum-induced flow, e.g., up to 4 μl/min for a 75 μm I.D. capillary, is observed. This vacuum-induced flow results in a significantly reduced efficiency in CE as it induces a parabolic rather than a plug-type flow profile. These effects could be minimized by decreasing the I.D. of the CE separation capillary down to 13 μm. The cathode end of the capillary is at the ion-source potential of 8 kV. A similar coaxial coupling for CF-FAB has been described by Suter and Caprioli [26].

The coaxial coupling for CF-FAB as developed by Moseley *et* al. [24,25] has been adapted for coupling CE to an electrospray interface [27,28]. As the outlet is at atmospheric pressure, conventional 75 μm I.D. CE separation capillaries can be used. A similar device has been described by Tsuji *et al.* [29]. A coaxial coupling for the ionspray interface has been described by Thibault and co-workers [30,31].

In a liquid-junction coupling the CE separation capillary and a transfer capillary to the interface are carefully aligned and the capillary ends are in close proximity. The gap between the two capillaries is of the order of 25 μm. The system is immersed in the make-up liquid. Minard and co-workers [12,32] were the first to describe a liquid-junction coupling to the CF-FAB interface. In the first design, the two capillaries were glued to a glass plate [12], whereas the second design had greater flexibility in mounting and aligning the capillaries [13]. Subsequently, a variety of liquid-junction coupling devices were described for CF-FAB interfacing [26,33–35]. The liquid-junction coupling, described by Henion's group [36] for use in combination with an ionspray interface, has been extensively used in a variety of CE-MS applications. A modified version of this

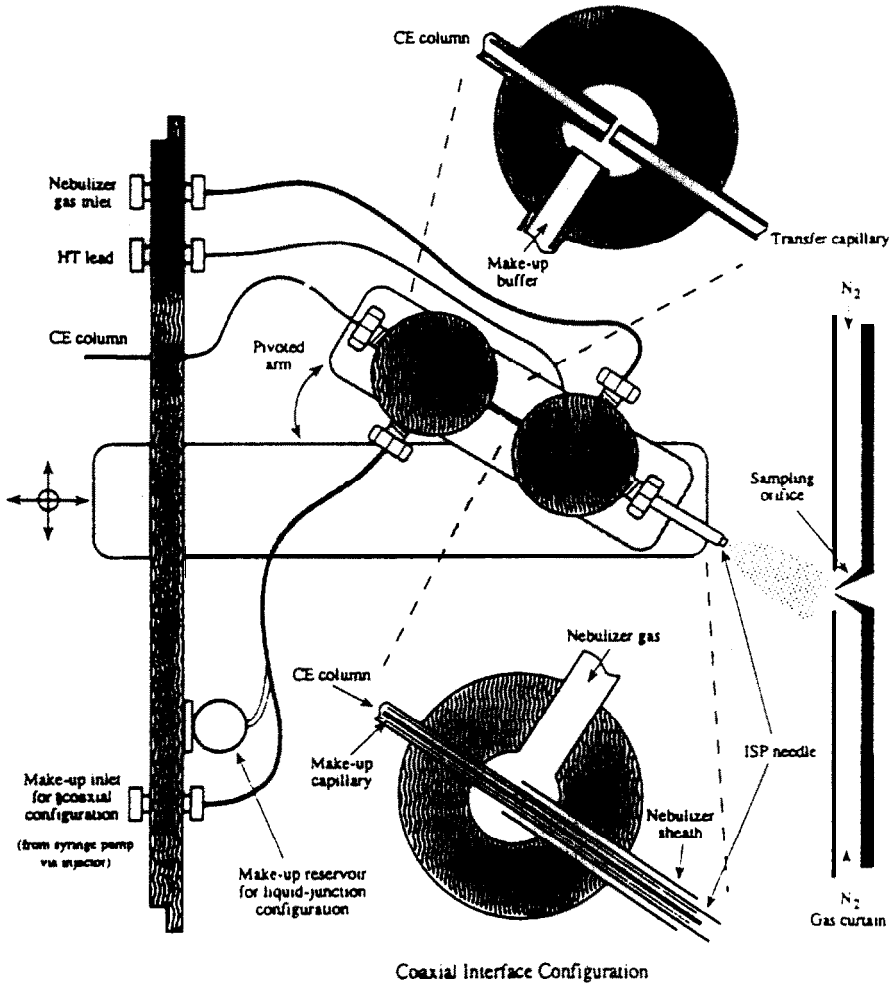

Fig. 3. Schematic diagram of the fully articulated ionspray interface for CE-MS showing both liquid-junction and coaxial configurations. From ref. 31 (© 1992, Elsevier).

device, as described by Pleasance *et al.* [31], is shown in Fig. 3, where both liquid-junction and coaxial couplings are illustrated.

Comparisons of the performances of liquid-junction and coaxial coupling were made by Suter and Caprioli [26] for a CF-FAB interface and by Pleasance *et al.* [31] for an ionspray interface. The liquid-junction coupling results in longer analysis times. According to Pleasance *et al.* [31], this cannot fully be explained by the additional length of the transfer capillary, but might be attributable to differences in the buffer composition at the coupling. The latter is also held responsible for the 10–12 μA higher current with the liquid-junction coupling. Suter and Caprioli [26] indicated that the longer retention time leads to an improved resolution, but it is questionable whether this effect can be exploited in solving separation problems.

The liquid-junction coupling was also found to give significant external peak broadening. Up to four times lower plate numbers were found for the liquid-junction coupling relative to the coaxial coupling in ionspray [31]. Even larger differences were reported for the CF-FAB system [26]. These losses are in agreement with values reported by Reinhoud *et al.* [34], who compared plate numbers

measured by on-capillary laser-induced fluorescence detection before and after the liquid-junction coupling. It can be calculated [31] that the peak broadening in the transfer capillary cannot fully account for this loss, meaning that in the actual coupling peak broadening also takes place.

With the ionspray system it was found that the make-up flow-rate in the liquid-junction coupling was lower than that with the coaxial coupling [31]. This results in a larger influence of the CE buffer system on the background and the noise. It may also influence the ionspray response in a compound-dependent way: 30–50% better responses were observed for some compounds in the coaxial system, but also a 30% better response for another compound [31] with the liquid-junction coupling. Significantly better signal-to-noise ratios in CF-FAB-MS were found for the coaxial relative to the liquid-junction coupling [26].

For coupling to the ionspray interface, coaxial coupling is to be preferred, as it shows greater flexibility and the ability for flow-injection analysis of compounds via the make-up liquid for tuning the system [31]. The same is true for coupling to electrospray interfaces. With the CF-FAB interface the situation is different: the necessity to work with 10 μm I.D. separation capillaries in order to reduce the vacuum-induced flow results in a significantly lower sample loadability. Further, the system is more difficult to construct and arcing through the narrow-bore capillaries may occur at higher field strengths [26].

4. SELECTED APPLICATIONS OF CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY

An overview of the compound classes analysed by CE-MS using various interfaces is given in Table 1. The major field of application in CE-MS is concerned with the analysis of peptides with special attention to neuropeptides and tryptic digests of larger proteins. Further, the analysis of quaternary ammonium salts and drugs is frequently reported. Some of these applications have been selected for a more detailed discussion, which is subdivided into qualitative and quantitative aspects.

4.1. *Qualitative analysis*

Because CE is extremely attractive as a separation method for biomolecules such as peptides and proteins, most of the papers are concerned with this field of application.

The analysis of chemotactic and neuropeptides was described by Moseley *et al.* [37]. The separation of six chemotactic peptides at the low femtomole

TABLE 1

APPLICATION OF CE-MS

References are given in which the analysis of the indicated compound classes with CE-MS using a particular interface is described.

Compound class	CF-FAB	Electrospray	Ionspray
Acid pesticides			36
Anthracyclines		62, 63	
Benzodiazepines			48
Quaternary ammonium compounds	34	8, 20–22	
Deoxynucleoside–PAH adducts[a]	35		
Macrolide antibiotics	28		31
Paralytic shellfish toxins	74		31, 75, 77
Peptides	12, 24-27, 32-34, 37, 45, 56, 74, 78	21, 23, 43, 44, 50, 51	16, 30, 36, 46, 49
Proteins		23, 29, 43, 44	30
Quaternary phosphonium salts		22	
Sulphonamides		47	48
Sulphonylureas			76
Sulphonated azo dyes			36, 79

[a] PAH = polycyclic aromatic hydrocarbon.

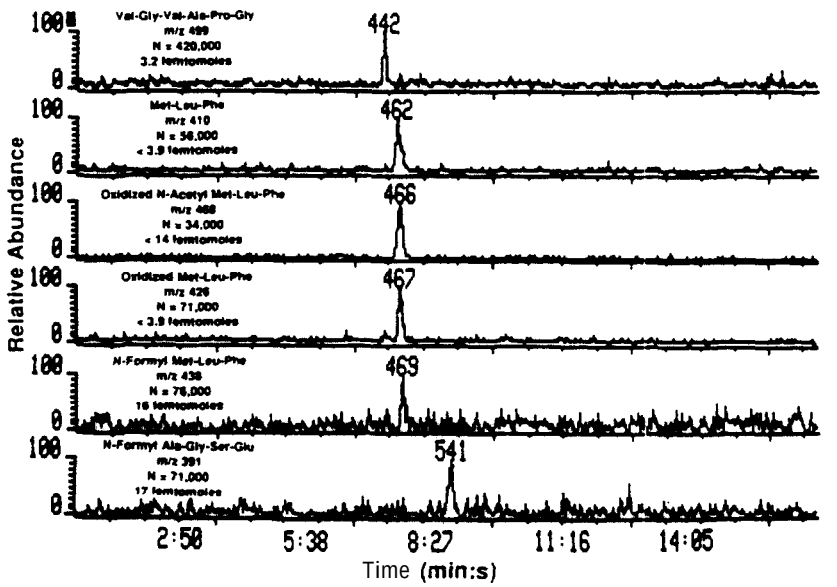

Fig. 4. Mass electropherograms for a mixture of chemotactic peptides. From ref. 37 (© 1991, American Chemical Society).

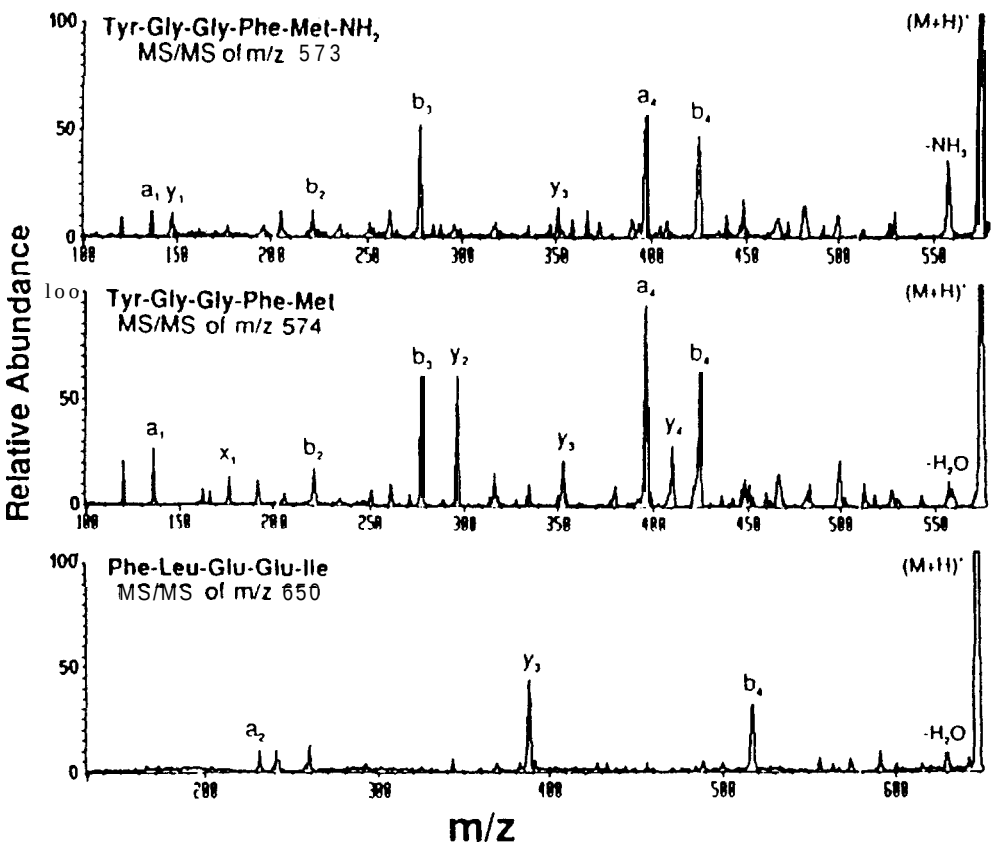

Fig. 5. Product-ion mass spectra of the CE-MS-MS analysis of three neuropeptides. From ref. 37 (© 1991, American Chemical Society).

level by CE-MS with coaxial coupling to a CF-FAB interface is shown in Fig. 4. The peptides were separated as negative ions at pH 8.5, but detected mass spectrometrically as positive ions using a CF-FAB matrix buffer of pH 3.5. Very high separation efficiencies ranging from 34 000 to 420 000 were achieved. The neuropeptides methionine-enkephalin, methionine-enkephalinamide and the pentapeptide FLEE1 were also analysed at the 20–40-fmol level under similar conditions. Product-ion spectra for the protonated molecules of these three compounds were acquired during CE-MS-MS (see Fig. 5). The spectra contain sequence-informative peaks, permitting identification of these peptides (except for FLEEI, for which the sequence information is incomplete).

The electrospray and the ionspray interfaces add another dimension to the MS analysis of peptides and proteins. The major impetus for the further development of this interface comes from the observation of series of multiply charged ions in the mass spectra of high-molecular-mass proteins, as first demonstrated by Fenn and co-workers [38,39]. As a mass spectrometer is separating and detecting the ions according to their *m/z* values, the multiple charging brings the peaks due to the protein within the mass range of a quadrupole mass spectrometer. From the ion envelope obtained in the mass spectrum, the average molecular mass of the protein can be calculated. Electrospray MS of peptides and proteins has been reviewed in various excellent papers [40–42].

The first to demonstrate the potential of the high-molecular-mass determination of proteins by CE–MS was Smith's group [23,43,44]. As an example, the separation of a mixture of horse heart cytochrome c (M_r 12 360), *Candida krusei* cytochrome c (M_r 12 548), whale myoglobin (M_r 17 199) and horse myoglobin (M_r 16 950) at the *ca.* 1 pmol per component level (0.1 mmol/l) in a 750 mm x 50 μm I.D. capillary at 25 kV is shown in Fig. 6. The detection of proteins at these low picomole levels has also been demonstrated by others with both electrospray [29] and ionspray [30]. For further characterization of the proteins, enzymic digests can be used. The CE-MS analysis of tryptic digests of proteins has been described by various workers, using either CF-FAB [33,45] or ionspray [30,36,46]. The peptide mixtures were analysed at the 10–50-pmol level.

The CE-MS analysis of sulphonamides [47,48] and benzodiazepines [48] using coaxial coupling to an electrospray interface [47] or liquid-junction coupling to an ionspray interface [48] may serve as examples of other compound classes analysed by CE-MS. In urine samples collected 2, 4 and 7 h after the oral administration of 30 mg of flurazepam, the N-1-hydroxyethyl and some minor metabolites of flurazepam were detected by CE-MS. The major metabolite was estimated to be present at the 4 μg/ml level [48].

4.2. *Quantitative analysis*

Quantitative analysis by CE-MS has hardly been described. A wide variety of compounds can be analysed at the picomole per injection level and in favourable instances even lower. Considering the small injection volume of only a few nanolitres, concentration detection limits in most instances are in the low-ppm range. This aspect is discussed in more detail in a later section.

The peaks obtained in selective ion monitoring in CE-MS from an aqueous dilution series of methionine-enkephalin ranging from 1 to 100 ppm are shown in Fig. 7 (1 ppm correspond to 15 pg or 30 fmol injected on to the capillary) [49]. However, in order to permit the analysis of this and other neuropeptides in cerebrospinal fluid, detection limits in the low-ppb range must be achieved.

Detection of adducts of nucleotides with polycyclic aromatic compounds using CE-MS with liquid-junction coupling and a CF-FAB interface has been described by Wolf *et al.* [35]. Selected reaction monitoring in MS-MS was applied to detect the DNA adducts at the 120-fmol level.

5. CAPILLARY ELECTROPHORESIS BUFFER COMPOSITION

In LC-MS operation, the user of non-volatile buffer constituents of the mobile phase is prohibited in routine use with all interface types. Considering the importance of the buffer type in CE, it is worthwhile to pay attention to the influence of buffer type on the performance of the CE-MS system. In general, it is thought that the use of non-volatile buffers is not as harmful in CE-MS as it is in LC-MS because of the dilution of the capillary

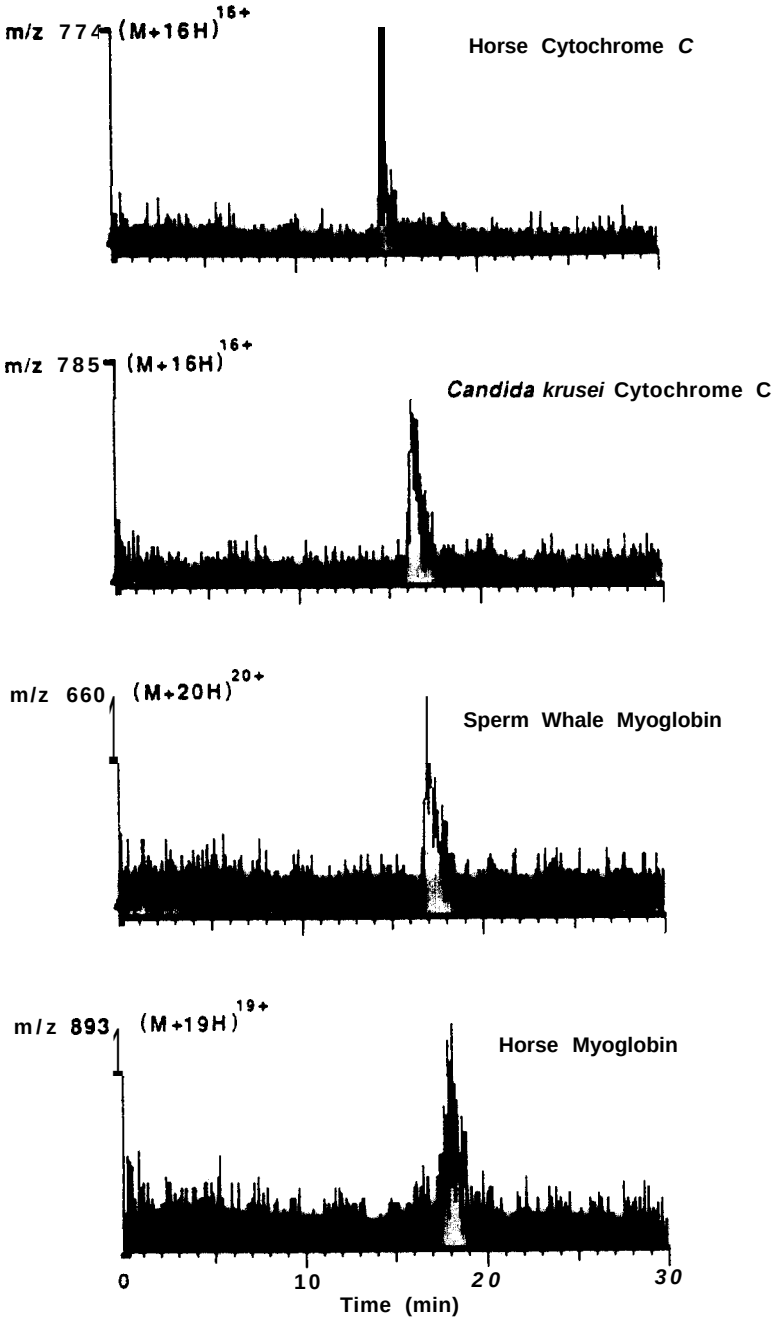

Fig. 6. Separation of a mixture of horse heart cytochrome *c* (M_r 12 360), *Candida krusei* cytochrome *c* (M_r 12 548), whale myoglobin (M_r 17 199) and horse myoglobin (M_r 16 950) at the *ca.* 1 pmol per component level in a 750 mm x 50 μm I.D. capillary at 25 kV. From ref. 44 (© 1989, Elsevier).

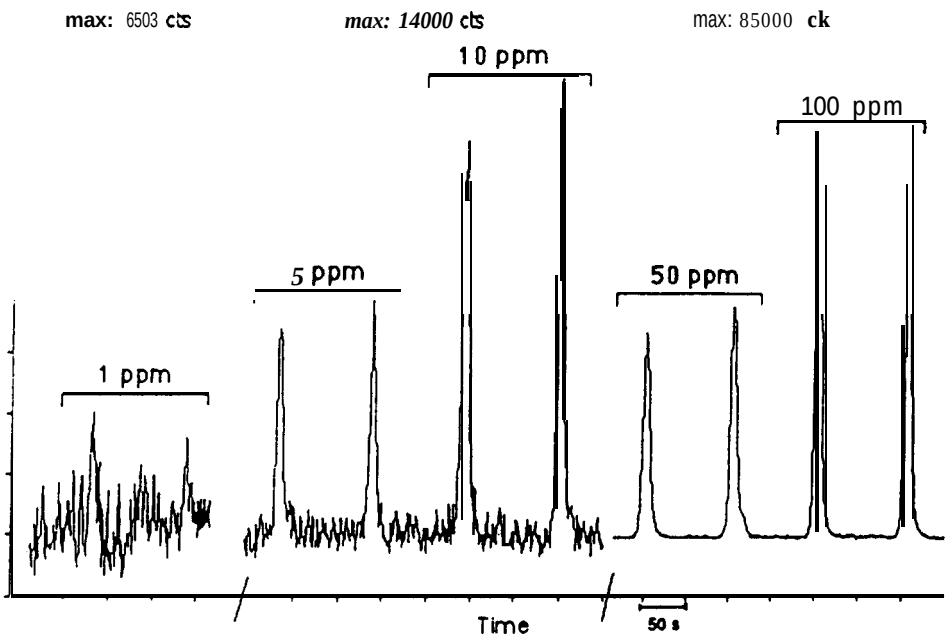

Fig. 7. CE-MS analysis of an aqueous dilution series of methionine-enkephalin on a 1000 mm x 100 μm I.D. capillary at 30 kV. Injection volume *ca.* 15 nl. From ref. 49 (© 1989, Elsevier).

effluent in the liquid-junction or coaxial coupling. The possible influence of the CE buffer on the ionization performance was briefly discussed in the context of a comparison of liquid-junction and coaxial couplings (see above) [31]. Various workers have considered the selection of the CE buffer to be used in CE-MS [31,33,50]. In most of the papers published on CE-MS, volatile buffer systems, *i.e.*, systems prepared with ammonium acetate, trifluoroacetic acid or formic acid have been used. The use of non-volatile buffers, e.g., containing phosphate, Tris or Trisma [21–23,31,43,44], has been described in only a few papers. Caprioli *et al.* [33] tested the addition of 50-150 mmol/l sodium chloride to the buffers in combination with a liquid-junction coupling and a CF-FAB interface. The sensitivity was significantly lower and abundant sodium adduct ions were detected. However, with 40 mmol/l sodium chloride and citric acid in the CE buffer and a volatile buffer system in the liquid-junction coupling, no detrimental effects on CF-FAB performance were experienced [33]. The influence of the buffer type and pH on the mass spectrometric detection of peptides and proteins has also been investigated, especially in combination with the electrospray interface. The separation of peptides as negative ions at high pH and MS detection as positive ions by using a low-pH make-up liquid has already been mentioned (*cf.*, Fig. 5). A more systematic study was described by Moseley *et al.* [50]. The CE separation of peptides at low pH, *i.e.*, a positive ions, is difficult because of interactions of the analytes with the negatively charged wall of the fused-silica capillary. However, low pH is favourable in the positive-ion MS detection of peptides because it promotes the protonation of the analytes. Moseley *et al.* [50] concluded that it is to be preferred to perform the separation at low pH in coated capillaries, in which wall interaction is avoided. CE-MS at low pH in coated capillaries was also described by Thibault *et al.* [30].

6. CRITICAL EVALUATION

Although CE-MS can still be considered to be an immature technique (most of the results reviewed in this paper have been acquired by only six research groups), the potential of the technique is clearly

demonstrated by the examples available in the literature. Determination of molecular mass and, by the use of MS-MS, even structure elucidation is obviously an attractive prospect when a high-efficiency separation method becomes available. Inherent to the improved separation power is the detection of new compounds, e.g., minor impurities. However, the MS characterization of these compounds requires sufficiently low detection limits with the CE-MS combination. At present, this is an aspect of major concern. As indicated in Section 4.2, the current minimum detectable concentrations are in the low-ppm range, despite the impressive absolute detection limits in the picomole or even femtomole range. A concentration range in, at least, the low-ppb range is required in most analytical applications of CE-MS.

Unlike, for instance, a UV detector which is concentration sensitive, the mass spectrometer is a mass flow-sensitive detector, which means that the response R(t) is directly proportional to the mass flow, dm/dt:

$$R(t) = \tau\,(dm/dt) = \tau\ C(t)\ F_{MS} \qquad (1)$$

where τ is the response factor, C(t) is the analyte concentration profile, i.e., a nearly Gaussian peak in LC-MS, and F_{MS} is the flow-rate to the mass spectrometer, which equals the flow-rate from the separation system multiplied by a split and/or dilution ratio S. Hence the response is determined by the mass or, better, the number of molecules per unit time coming from the separation system, introduced into the ion source and arriving at the detector. As an example, virtually the same absolute detection limits are found in coaxial coupling to CF-FAB with 10 μm I.D. capillaries and liquid-junction coupling to CF-FAB with 75 μm I.D. capillaries (e.g., compare refs. 33 and 24). Nevertheless, it has to be realized that at constant flow-rate for a mass flow-sensitive system the response is directly proportional to the concentration.

At various conferences and symposia it has been argued that with the electrospray and ionspray interfaces the mass spectrometer acts as a concentration-sensitive detector, which does not imply that it *is* a concentration-sensitive detector. Moreover, as is described below, the (highly attractive) concentration-sensitive behaviour is not at all demonstrated in CE-MS. Apparent concentration sensitivity can be attributed to the fact that splitting takes place at the ion-sampling orifice in the atmospheric-pressure ion source, i.e., at the nozzle or the glass capillary, where the ions are introduced into the high-vacuum part of the instrument. Concentration rather than mass flow sensitivity looks extremely promising and attractive in CE-MS, because as a result of the excellent separation efficiencies the sample is offered by CE to the mass spectrometer in a narrow high-concentration band. Although as a result of the poor loadability the number of molecules in such a band is small, the concentration is comparable to that in chromatography or even better. Smith et al. [51] stated that nearly 100% ionization efficiency can be achieved in electrospray ionization, but that only *ca.* 1% of the ions are entering through the nozzle. Further, the electrospray and ionspray systems are space-charge limited ion sources. Busman et al. [52] showed that in such a device the ion density in the aerosol is limited by the space charge, which means that only a limited number of ions can be produced in a certain volume and no concentrating or focusing effects of ions towards the ion sampling orifice can be achieved. At a certain concentration, typically a concentration exceeding 10^{-4} mol/l for a singly charged species [51], saturation will occur and the signal no longer increases with increasing sample concentration [53,54]. The ion sampling orifice collects a fixed volume from the source. As a consequence, under these conditions of saturation the electrospray ion source is relatively insensitive to the flow-rate, of course within the limits of stable electrospray performance (*cf.*, refs. 51 and 53).

In the present discussion, attention must be focused on the low concentrations rather than on the saturation effects. From the literature, it is not yet clear what happens to the spray plume shape, and thus to the ion concentration in the aerosol, when the sample concentration is decreased. Most likely, and especially in CE-MS operation, the spray plume is actually sustained by the buffer electrolyte and is not much influenced by the analyte present. In a buffer-sustained spray, the analyte concentration decreases with a decrease in the number of analyte molecules eluting from the inlet capillary. A decrease of the number of analyte molecules is the inevitable result of miniaturization of the inlet system, e.g., on going from a l-mm I.D. microbore LC column to a 100-μm I.D. CE separation capil-

lary. Therefore, because the analyte concentration in the spray decreases, the number of analyte ions introduced into the mass spectrometer also decreases. Irrespective of the (supposed) concentration-sensitive behaviour of the electrospray, the mass spectrometer responds to the number of ions that reach the detector. Therefore, poor loadability results in a poor response, as expected from a mass flow-sensitive device. A good demonstration of this phenomenon is provided in a paper on the determination of enkephalins in equine cerebrospinal fluid cited in Section 4 [49]. Mück and Henion [49] compared the concentration detection limits that can be achieved in micro-LC-MS using a l-mm I.D. column and a 5-μl injection and in CE-MS using a 100 μm I.D. capillary and a 15–25-nl injection. For LC-MS a limit of detection of 20 ppb (100 pg or 200 fmol) was reported, which is sufficiently low for practical applications, whereas for CE-MS a limit of detection of 2 ppm (20 pg or 60 fmol) was found. Despite the fact that CE results in a higher concentration in the peak (the peak from LC and CE are found to be of approximately the same width, whereas the peak volume differs by a factor of at least 100) and the ionspray performance is improved at the 10-20 μl/min in CE-MS relative to the 40 μl/min in LC-MS [49], the decrease in concentration detection limits matches the decrease in loadability. This is in full agreement with the mass flow sensitivity of the detection system.

Currently, various solutions to this problem are under investigation. Instrumental developments at the electrospray ion source may lead to better performance, *i.e.*, improved overall ionization efficiency [51,55].

Another approach is the use of array detection systems in mass spectrometry that show an improved performance over the conventional electron multiplier. The improvements that can be achieved with a so-called PATRIC array detector, which is commercially available on a Finnigan MAT 900 instrument, have been demonstrated by Reinhoud et al. [56]. As an example, the mass spectra obtained in the CE-MS analysis of some β-endorphins with either the electron multiplier or the PATRIC array detector in the static mode are compared in Fig. 8.

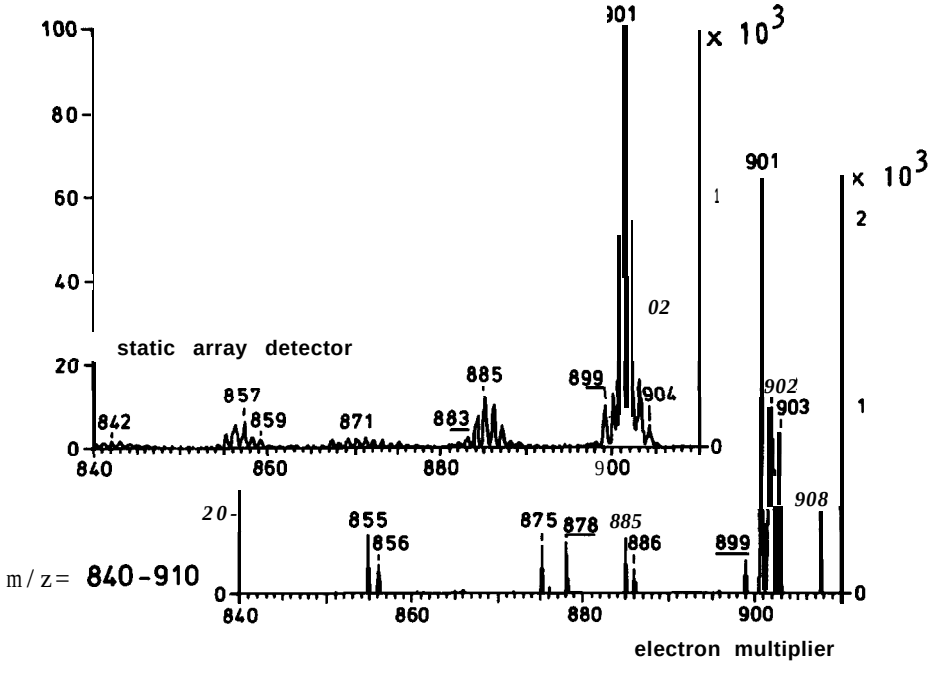

Fig. 8. Improved spectrum. quality in the CE-MS analysis of a β-endorphin 8-15 fragment by using static PATRIC array detection instead of electron multiplier. CE-MS with liquid-junction coupling to a CF-FAB interface. From ref. 9 (© 1992, Marcel Dekker).

These results indicate that a 100–1000-fold improvement in detection limit is possible by the use of an array detector.

A third approach towards improved concentration detection limits is the on-line combination of isotachophoresis (ITP) and capillary electrophoresis. In ITP, two buffer systems are selected that have a higher and a lower electrophoretic mobility than the sample constituents. The sample is introduced between these two buffers. The two buffers bracket the sample components as leading and trailing electrolytes. As a result of the applied axial electric field, sample components with different electrophoretic mobilities are separated into sharp bands, the length of which is proportional to the amount of analyte. ITP can have distinct advantages over CE. Larger sample volumes can be introduced. During a separation the various ions in the mixture are arranged in bands of decreasing mobility, the trailing electrolyte having the lowest mobility. Finally, a steady state is reached with each analyte in its own band and all bands moving with the same velocity. If the analyte in the sample solution is more dilute than the leading buffer, the analyte is actually concentrated as it separates in its own band. The ionic concentrations of each band are equal; different amounts of analyte in the original sample are reflected in the length of the analyte band. Thus, a plateau in sample concentration is reached in ITP instead of the very narrow peak that is achieved in CE.

The use of ITP in sample pretreatment for CE has been described by various groups [57–61]. Up to 1000-fold improvements in concentration detection limits have been demonstrated [57,58]. A schematic diagram of the experimental set-up for ITP-CE-MS is shown in Fig. 9. The rationale of the combined ITP-CE approach for application in CE-MS is that the initial sample concentration can be adapted to the concentration required in CE-MS. A 200-fold improvement in concentration detection limit for the ITP-CE-MS over the CE-MS analysis of anthracyclines has been demonstrated [62,63]. Obviously, other concentrating sample pretreatment methods may be used to tune optimally the analyte concentration to the needs of CE-MS. In that respect, the possibilities of zone sharpening must be mentioned. In zone sharpening, ion-neutral separation and concentration effects during electrokinetic sample injection into the CE capillary are applied for concentrating analytes on top of the CE separation capillary [64,65].

7. OTHER ELECTROMIGRATION TECHNIQUES COUPLED TO MASS SPECTROMETRY

In addition to CE, several other electromigration techniques have been combined with mass spectrometry. These combinations are briefly discussed in this section.

The on-line combination of ITP and electrospray

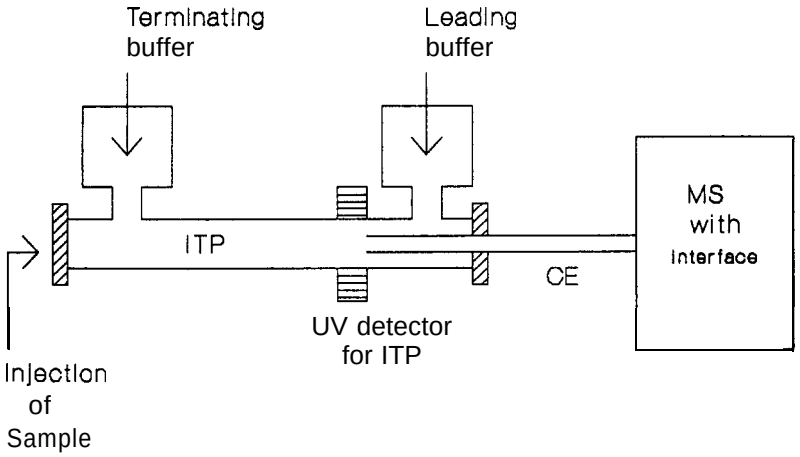

Fig. 9. Schematic diagram of the combined isotachophoresis (ITP)–capillary electrophoresis (CE)–mass spectrometry (MS) system.

MS has been described by Smith and co-workers [44,66]. The separations were developed in 100 μm I.D. fused-silica capillaries. The technique was applied to the separation of quaternary ammonium and phosphonium salts, amines and amino acids [66] and to the analysis of peptides, such as bradykinin, angiotensin, cytochrome c and myoglobin [44]. The combination of higher sample loadability, the concentrating effect and the concentration plateau, characteristic of an ITP separation, make ITP-MS especially useful in structure elucidation, e.g., peptide sequencing using MS-MS.

The combination of gel-filled CE and MS was described by Garcia and Henion [67]. The gel-filled capillary was connected via a liquid-junction coupling to an ionspray interface. Relatively high concentrations of urea and other buffers do not elute from the gel, whereas the separated analytes are successfully transported to the mass spectrometer. On-line gel-tilled CE-MS analysis of mixtures of aromatic sulphonates, dansylated amino acids and polyacrylic acids- at low-picomole levels has been demonstrated [67].

The combination of pseudo-electrochromatography and MS has been described. Pseudo-electrochromatography involves a pressure-driven solvent flow through a packed column to which an axial electric field is applied. The main feature of the applied field is the ability to tune the selectivity in a separation of ionic compounds under MS-compatible conditions. Separation and MS detection of alkaloids and nucleotides with a CF-FAB interface [68] and of aromatic glucuronides and food dyes with an electrospray interface [69] have been demonstrated.

8. OTHER APPROACHES TO CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY

The major attention in this review was focused on the most widely used CE-MS approaches. Several other approaches, both on-line and off-line, have been described.

The coupling of CE to a ion-mobility spectrometer was described by Hallen *et al.* [70]. Various interfaces based on ESP phenomena were tested, *i.e.*, a nebulization-assisted spray interface, a flow-assisted spray interface and a direct-coupled spray interface. Preliminary data were given for quaternary ammonium salts [70].

Takigiku and co-workers described the off-line combination of CE and plasma desorption [71] and matrix-assisted laser desorption [72] MS. In the plasma desorption experiment, fractions from the CE were directly deposited on nitrocellulose foils, which were subsequently transferred to the target holder of the plasma desorption mass spectrometer and measured. Data were presented for low-picomole amounts of various peptides, such as bradykinin, insulin and a-lactalbumin. Detection limits of 0.25 pmol were reported for bradykinin and 5 pmol for lactalbumin [71]. For laser desorption fractions were collected in a 1–2-μl polypropylene tube, where they were mixed with sinapinic acid, the matrix for laser desorption. A detection limit of 50 fmol was reported for lactalbumin, representing a 100-fold improvement relative to plasma desorption MS [72]. The coupling of CE and matrix-assisted laser desorption MS has also been described by Van Veelen *et al.* [73]. The effluent from the CE capillary was deposited as small drops with the use of a sheath flow containing 2,5-dihydroxybenzoic acid, the matrix for laser desorption, on a discontinuously moving belt. The belt surface was then introduced into a Finnigan MAT Vision 2000 laser desorption mass spectrometer, which allows the selection of different sites on the target for laser desorption by means of an x-y manipulator. In this way, a mixture of β-endorphins could be analysed at the 100-fmol level, while actually maintaining the electrophoretic resolution [73].

9. CONCLUSIONS AND PERSPECTIVES

The on-line combination of CE and MS is a very young technique, the first paper appearing in April 1987 [8]. The technique has been evolving very rapidly since then, and the results are nowadays very impressive. However, the major problem that must be solved to allow a broad breakthrough of CE-MS as an analytical tool is related to the concentration detection limits achievable. The femtomole detection limits demonstrated are indeed very impressive, although they correspond to concentrations in the nmol/ml range (μg/ml for compounds with a molecular mass of 1000) owing to the injection volumes of *ca.* 1 nl. These concentrations and amounts might be feasible in some biochemical applications, for instance related to recombinant peptide and protein

products, but not in, for instance, bioanalytical and environmental problems. The use of array detection in MS and of preconcentration prior to CE, e.g., by means of on-line ITP, is advocated to solve these problems.

Also of importance is the fact that the high-efficiericy separations in CE result in very narrow peaks which can pose high demands on the scanning rates in MS, especially in structure elucidation where full scan acquisition is obligatory. CE is preferentially applied in peptide- and protein-related studies, leading to relatively high mass-to-charge ratios in electrospray and ionspray and especially in CF-FAB work. Scan times of at least 1 s are needed to obtain a reliable spectrum over a mass range *m/z* 200–2000 on a quadrupole instrument. For sector instruments the situation is even worse. Scan times play an even larger role in MS-MS work.

Finally, micellar-based electromigration approaches have been shown to provide unmatched separation power, but MS detection has not yet been achieved in such systems. This certainly is one of the important challenges of current and future research.

REFERENCES

1 0. Vesterberg, *J. Chromatogr., 480 (1989) 3.*
2 K. D. Altria, M. M. Rogan and G. Finlay, *Chromatography and Analysis, 1990, p. 9.*
3 J. W. Jorgenson and K. D. Lukacs, *J. Chromatogr., 218 (1981) 209.*
4 J. W. Jorgenson and K. D. Lukacs, Science, 22 (1983) 266.
5 S. F. Y. Li, *Capillary Electrophoresis. Principles, Practice and Applications (Journal of Chromatography Library,* Vol. 52), Elsevier, Amsterdam, 1992.
6 R. A. Wallingford and A. G. Ewing, *Adv. Chromatogr., 29 (1989)* 1.
7 W. G. Kuhr and C. A. Monnig, *Anal.* Chem., 64 (1992) 389R.
8 J. A. Olivares, N. T. Nguyen, C. R. Yonker and R. D. Smith, *Anal.* Chem., 59 (1987) 1230.
9 W. M. A. Niessen and J. van der Greef, *Liquid Chromatography Mass Spectrometry,* Marcel Dekker, New York, 1992.
10 W. M. A. Niessen, U. R. Tjaden and J. van der Greef, *J. Chromatogr., 554 (1991) 3.*
11 R. M. Caprioli (Editor), *Continuous-Flow Fast-Atom Bombardment,* Wiley, New York, 1990.
12 R. D. Minard, D. Chin-Fatt, P. Curry, Jr. and A. G. Ewing, presented at the *36th ASMS Conference on Mass Spectrometry and Allied Topics, June 5–10, 1988, San Francisco, CA,* ASMS, Santa Fe, NM, p. 950.
13 C. M. Whitehouse, R. N. Dreyer, M. Yamashita and J. B. Fenn, *Anal. Chem.*, 57 (1985) 675.
14 A. P. Bruins, T. R. Covey and J. D. Henion, *Anal.* Chem., 59 (1987) 2642.
15 A. P. Bruins, *Mass Spectrom. Rev.,* 10 (1991) 53.
16 E. D. Lee, W. M. Mück, J. D. Henion and T. R. Covey, *J. Chromatogr., 458 (1988) 313.*
17 W. M. A. Niessen and H. Poppe, *J. Chromatogr., 394 (1987) 21.*
18 W. M. A. Niessen and H. Poppe, *J. Chromatogr., 323 (1985) 37.*
19 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.
20 R. D. Smith and H. R. Udseth, *Nature,* 331 (1988) 639.
21 R. D. Smith, J. A. Olivares, N. T. Nguyen and H. R. Udseth, *Anal.* Chem., 60 (1988) 436.
22 R. D. Smith, C. J. Barinaga and H. R. Udseth, *Anal.* Chem., 60 (1988) 1948.
23 J. A. Loo, H. R. Udseth and R. D. Smith, *Anal. Biochem., 179 (1989) 404.*
24 M. A. Moseley, L. J. Deterding, K. B. Tomer and J. W. Jorgenson, *Rapid Commun.* Mass *Spectrom., 3 (1989) 87.*
25 M. A. Moseley, L. J. Deterding, K. B. Tomer and J. W. Jorgenson, *J. Chromatogr., 480 (1989) 197.*
26 M. J.-F. Suter and R. M. Caprioli, *J. Am. Soc. Mass Spectrom., 3 (1992) 198.*
27 L. J. Deterding, C. E. Parker, J. R. Perkins, M. A. Moseley, J. W. Jorgenson and K. B. Tomer, *J. Chromatogr., 554 (1991) 329.*
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.*
29 K. Tsuji, L. Bacyznskyj and G. E. Bronson, *Anal.* Chem., 64 (1992) 1864.
30 P. Thibault, C. Paris and S. Pleasance, *Rapid Commun. Mass Spectrom., 5 (1991) 484.*
31 S. Pleasance, P. Thibault and J. Kelly, *J. Chromatogr., 591 (1992) 325.*
32 R. D. Minard, D. Luckenbill, P. Curry, Jr. and A. G. Ewing, *Adv. Mass Spectrom.,* 11 (1989) 436.
33 R. M. Caprioli, W. T. Moore, M. Martin, B. B. DaGue, K. Wilson and S. Morning, *J. Chromatogr., 480 (1989) 247.*
34 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.*
35 S. M. Wolf, P. Vouros, C. Norwood and E. Jackim, *J. Am. Soc. Mass Spectrom., 3 (1992) 757.*
36 E. D. Lee, W. M. Mück, J. D. Henion and T. R. Covey, *Biomed. Environ. Mass Spectrom., 18 (1989) 844.*
37 M. A. Moseley, L. J. Deterding, K. B. Tomer and J. W. Jorgenson, *Anal.* Chem., 63 (1991) 109.
38 C. K. Meng, M. Mann and J. B. Fenn, presented at the *36th ASMS Conference on Mass Spectrometry and Allied Topics, June 5–10, 1988, San Francisco, CA,* ASMS, Santa Fe, NM, p. 771.
39 M. Mann, C. K. Meng and J. B. Fenn, *Anal.* Chem., 61 (1989) 1702.
40 J. B. Fenn, M. Mann, C. K. Meng, S F. Wong and C. M. Whitehouse, *Mass Spectrom. Rev.,* 9 (1990) 37.
41 M. Mann, Org. Mass *Spectrom., 25 (1990) 575.*
42 R. D. Smith, J. A. Loo, R. R. Ogorzalek Loo, M. Busman and H. R. Udseth, *Mass Spectrom. Rev.,* 10 (1991) 359.
43 J. A. Loo, H. K. Jones, H. R. Udseth and R. D. Smith, *J. Microcol. Sep.,* 1 *(1989) 223.*

44 R. D. Smith, J. A. Loo, C. J. Barinaga, C. G. Edmonds and H. R. Udseth, *J.* Chromafogr., 480 (1989) 211.
45 M. J.-F. Suter, B. B. DaGue, W. T. Moore, S.-N. Lin and R. M. Caprioli, *J.* Chromarogr., 553 (1991) 101.
46 I. M. Johansson, E. C. Huang, J. D. Henion and J. Zweigenbaum, *J.* Chromatogr., 554 (1991) 311.
47 J. R. Perkins, C. E. Parker and K. B. Tomer, *J.* Am. *Soc. Mass* Spectrom., 3 (1992) 139.
48 I. M. Johansson, R. Pavelka and J. D. Henion, *J. Chromatogr.*, 559 (1991) 515.
49 W. M. Mück and J. D. Henion, *J.* Chromatogr., 495 (1989) 41.
50 M. A. Moseley, J. W. Jorgenson, J. Shabanowitz, D. F. Hunt and K. B. Tomer, *J.* Am. *Soc. Mass* Spectrom., 3 (1992) 289.
51 R. D. Smith, J. A. Loo, C. G. Edmonds, C. J. Barinaga and H. R. Udseth, *J.* Chromatogr., 516 (1990) 157.
52 M. Busman, J. Sunner and C. R. Vogel, *J.* Am. *Soc. Mass* Spectrom., 2 (1991) 1.
53 M. G. Ikonomou, A. T. Blades and P. Kebarle, ***Anal.*** Chem., 62 (1990) 957.
54 M. G. Ikonomou, A. T. Blades and P. Kebarle, ***Anal.*** Chem., 63 (1991) 1989
55 R. D. Smith, H. R. Udseth, C. J. Barinaga and C. G. Edmonds, *J.* Chromatogr., 559 (1991) 197.
56 N. J. Reinhoud, E. Schröder, U. R. Tjaden, W. M. A. Niessen, M. C. ten Noever de Brauw and J. van der Greef, *J.* Chromatogr., 516 (1990) 147.
57 D. S. Stegehuis, H. Irth, U. R. Tjaden and J. van der Greef, *J.* Chromatogr., 538 (1991) 393.
58 D. S. Stegehuis, U.R. Tjaden and J. van der Greef, *J.* Chromatogr., 591 (1992) 341.
59 D. Kaniansky and J. Marak, *J.* Chromatogr., 498 (1990) 191.
60 F. Foret, V. Sustacek and P. Bocek, *J.* Microcol. *Sep.*, 2 (1990) 229.
61 F. Foret, E. Szoko and B. L. Karger, ***J. Chromatogr., 608 (1992) 3.***
62 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.***
63 ***N.*** J. Reinhoud, A. P. Tinke, U. R. Tjaden, W. M. A. Niessen and J. van der Greef, ***J. Chromatogr., 627 (1992) 263.***
64 R.-L. Chien and D. S. Burgi, ***Anal.*** Chem., 64 (1992) 489A.
65 N. J. Reinhoud, U. R. Tjaden, H. Irth and J. van der Greef, ***J. Chromatogr., 574 (1992) 327.***
66 H. R. Udseth, J. A. Loo and R. D. Smith, ***Anal.*** Chem., 61 (1989) 228.
67 F. Garcia and J. D. Henion, ***Anal.*** Chem., 64 (1992) 985.
68 E. R. Verheij, U. R. Tjaden, W. M. A. Niessen and J. van der Greef, ***J. Chromatogr.,*** submitted for publication.
69 M. Hugener, W. M. A. Niessen, U. R. Tjaden and J. van der Greef, ***J. Chromatogr.,*** submitted for publication.
70 R. W. Hallen, C. B. Shumate, W. F. Siems, T. Tsuda and H. H. Hill, Jr., ***J. Chromatogr., 480 (1989) 233.***
71 R. Takigiku, T. Keough, M. P. Lacey and R. E. Schneider, ***Rapid Commun. Mass Spectrom., 4 (1990) 24.***
72 T. Keough, R. Takigiku, M. P. Lacey and M. Purdon, ***Anal.*** Chem., 64 (1992) 1594.
73 P. A. van Veelen, U. R. Tjaden, J. van der Greef, A. Ingendoh and F. Hillenkamp, ***J. Chromatogr.,*** in press.
74 L. J. Deterding, M. A. Moseley, K. B. Tomer and J. W. Jorgenson, ***J. Chromatogr., 554 (1991) 73.***
75 P. Thibault, S. Pleasance and M. V. Laycock, ***J. Chromatogr., 542 (1991) 483.***
76 F. Garcia and J. D. Henion, ***J. Chromatogr., 606 (1992) 237.***
77 ***S.*** Pleasance, S. W. Ayer, M. V. Laycock and P. Thibault, ***Rapid Commun. Mass Spectrom., 6 (1992) 14.***
78 M. A. Moseley, L. J. Deterding, K. B. Tomer and J. W. Jorgenson, ***J. Chromatogr., 516 (1990) 167.***
79 E. D. Lee, W. M. Mück, J. D. Henion and T. R. Covey, ***Biomed. Environ. Mass Spectrom., 18 (1989) 253.***