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Abstract

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COUPLING CE AND MICROCHIP-BASED DEVICES WITH MASS SPECTROMETRY

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ABSTRACT

Recent developments in capillary electrophoresis coupled to mass spectrometry (CE–MS) are reviewed from over 400 references covering the significant challenges, issues, and results obtained with CE–MS analyses.

A particular attention is paid to instrumental aspects for successful hyphenation of CE with MS, regarding interfaces and ionization sources. A special section is dedicated to quantitative results, and various methodologies to achieve sensitive and repeatable analysis are considered.
Dedicated applications of capillary zone electrophoresis (CZE) coupled to MS are discussed, particularly in the field of drug analysis. Development of other capillary-based electrodriven separation techniques such as non-aqueous capillary electrophoresis (NACE), micellar electrokinetic chromatography (MEKC), and capillary electrochromatography (CEC) hyphenated with MS are also treated. The successful coupling of these electromigration schemes with MS detection provides an efficient and sensitive analytical tool for the separation, quantitation, and identification of numerous pharmaceutical, biological, therapeutic, and environmental compounds.

Chip-based microdevices are finally discussed, regarding fabrication methods, designs, MS interfacing, and applications. Current capabilities and limitations for future use are emphasized considering improvements in methodology and instrumentation.

I. INTRODUCTION

Capillary electrophoresis (CE) is currently a powerful separation technique and has found numerous applications in various analytical fields. Several advantages such as a high-efficiency rapid method development, simple instrumentation, and low sample consumption are the main reasons for this success. UV-Vis spectrophotometry is probably the most widely used detection technique with CE because of the simplicity of the on-line configuration. However, its sensitivity, directly related to the optical pathlength afforded by the internal diameter of capillaries, which is in the μm range, is low and remains the major bottleneck of this technique. UV detection thus requires relatively high analyte concentrations, and is often unsuitable for numerous applications such as the bioanalytical field or impurities’ peak profiling. Therefore, other detectors are required for the analysis of complex mixtures and many detection techniques have been already hyphenated with CE such as laser-induced fluorescence (LIF) and electrochemical (EC) detections. Because pharmaceutical and chemical structures do not always possess a strong chromophore or fluorophore, a derivatization procedure is often mandatory for a sensitive spectroscopic detection while EC is limited to electroactive substances. In this context, the on-line combination of high-efficiency CE separations and mass spectrometry (MS) is an attractive perspective and presents some major benefits. Among them, it enhances sensitivity and enables determination of co-migrating compounds with different mass-to-charge ratios (m/z). MS provides a higher potential for an unambiguous identification and confirmation of components in complex mixtures, and potentially gives some information concerning the structure of the separated compounds. Therefore, due to its high sensitivity and specificity, MS is a detector of choice for CE, and CE–MS coupling provides a powerful combination for performing rapid, efficient, and sensitive analysis.

The present chapter will review instrumental aspects for successful coupling of CE with MS, regarding interfaces, ionization sources, and analyzers. Practical considerations concerning different CE modes such as CZE, NACE, MEKC, and CEC coupled with MS will also be discussed and illustrated with a focus on recent pharmaceutical applications. Additionally, quantitative CE–MS will be presented and various methodologies used to achieve sensitive and repeatable analysis will be discussed. Finally, the final section of this chapter will give an overview on new devices (i.e., microchips), hyphenated to MS, in terms of fabrication methods, microchip designs, MS interfacing, and applications.

II. CE–MS COUPLING AND INSTRUMENTATION

Since the introduction in the mid-1980s, different MS systems and ionization interfaces have been described.1–8 In the last few years, CE–MS techniques were successfully applied in a variety of fields with commercially available setups. Today, manufacturers propose user-ready CE–MS, and the technique can be routinely employed in numerous dedicated fields of
analytical chemistry. Technological developments in instrumentation continue to occur, and improvements in homebuilt instruments are regularly reported, which offer advantages in terms of reduced cost, capillary dimension, applied voltage, and interface modifications.

Several approaches have been investigated for CE–MS coupling. Most of the developed interfaces were initially used for LC hyphenation and adapted to the constraints of CE analysis. In establishing on-line CE–MS interfacing, several difficulties have to be considered.

(i) The electrical connection at the interface side of the separation capillary must be achieved with the cathode end of the capillary directly connected to the MS interface. (ii) The typical flow rate in CE capillary, resulting from the electroosmotic flow (EOF), does not exceed 100 nL/min and is generally not compatible to conventional LC–MS interfaces. Therefore, either a make-up liquid or a miniaturized electrospray system should be implemented. (iii) The presence of nonvolatile constituents included into the background electrolyte (BGE), such as selectivity modifier additives, may be detrimental to the MS performance, owing to ion source or analyzer contamination.

A. Interfaces

As indicated above, modified LC–MS interfaces are generally applied in CE–MS. In this section, attention is focused on the coupling devices that ensure an adequate operation of CE–MS interfacing. Two configurations are conventionally distinguished, with the addition of a make-up liquid or without additional liquid, in a miniaturized interface. A growing interest in the development and use of new sheathless interfaces occurs according to its improved sensitivity over the sheath-flow approach. However, due to its instrumental simplicity, versatility, and robustness, the coaxial sheath-flow interface still represents the most common approach when hyphenating CE with MS. With these interfaces, the electrical connection and flow rate compatibility issues are resolved, but a special care must be taken to avoid any loss in CE efficiency due to external peak broadening.

I. Sheath-Flow Interfaces

In addition to the electrical connection and flow rate issues, the analytes must be released from solvent molecules and brought into the gas phase. Liquid flow rates in conventional CE suggest that evaporation is easy but droplets can be formed at the tip of the CE capillary, which causes an unstable flow to the mass analyzer. To overcome this problem, make-up flows are often used. Although the sheath liquid system is relatively easy to implement and use, it is rather demanding in terms of optimizing the operational parameters to obtain a stable and repeatable spray (e.g., capillary-tip position, sheath liquid flow rate, and composition). Two types of sheath-flow interfaces can be distinguished: the coaxial sheath liquid interface and the liquid-junction interface. The distinction between both interfaces is based on the integration of the make-up liquid: In the coaxial configuration, liquid addition takes place proximal to the MS orifice while the liquid-junction geometry provides the make-up liquid distal to the sprayer tip.

(a) Coaxial Sheath-Flow Interface:

With the coaxial interface, initially reported by Smith et al., the problem of irregular droplets formation is circumvented by using a sheath flow in the μL/min range surrounding the CE capillary and mixing with the CE buffer at the tip of the separation capillary. It provides electrical contact at the outlet end of the separation capillary, appropriate flow, and solvent conditions for ionization and evaporation, independent on the nature of the CE buffer solution. It is the most commonly used interface and can be easily implemented. Furthermore,
high buffer-salt concentrations, which have unintended negative effects on the efficiency of the spray, are diluted with the sheath liquid, and spray stability is usually improved. Dilution of the CE effluent by the sheath liquid flow rate does not significantly affect the sensitivity of the detection since the sheath liquid is also evaporated during the spray process. A number of papers have described sheath liquid interface optimization and practical aspects in setting up a coaxial CE–MS interface.21–26 A typical coaxial sheath-flow interface is depicted in Figure 1.

(b) Liquid-Junction Interface:
This type of interface was first developed by Henion and co-workers27,28 and further modified by different authors.21,29–31 The electrical connection is established at the junction between the CE capillary and a second one leading to the mass spectrometer. In an open connection, the junction of both capillaries is usually placed under a liquid reservoir together with the electrode. This liquid serves as make-up flow and carries the analytes toward the detector both with the siphoning action of the field generated by the source potential and with the aspirating effect of the nebulizing gas. Because the liquid-junction interface is partially disconnected from the emitter (both physically and electrically), the latter can be easily replaced and problems related to the emitter are isolated from those linked to the separation capillary. However, the gap between both capillaries is a critical element and can introduce significant external peak broadening. Therefore, an extreme care must be taken for proper alignment.

2. Sheathless Interfaces
Several reports concerning the development of stable and rugged sheathless interfaces were proposed.32,33 The first sheathless interface was developed by Olivares et al.,19 and two types of sheathless interfaces are currently distinguished. The first one consists of a nanospray needle, which is inserted with a connection unit to the CE capillary. This setup allows changing the spray needle alone independently on the capillary exchange.26,34–40 The second approach involves the use of the end of capillary tip as an emitter with the help of a capillary-outlet conductive coating41–53 or by inserting a conductive wire into the capillary outlet.54–56 This type of device is usually homebuilt, and different procedures for modifying the capillary tip and applying the conductive layer are reported.57–64

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**FIGURE 1** Experimental setup for CE–ESI/MS with a coaxial sheath-flow interface.
The main advantage of sheathless interfaces is their high sensitivity compared to sheath-flow interfaces. First, a larger portion of produced ions reaches the MS because the nanospray tip is commonly positioned more closely to the MS orifice than other interfaces; second, the smaller droplets obtained due to the reduced flow rate improve analyte ionization; finally, no sample dilution by the additional sheath flow occurs. Furthermore, compatibility with the low flow rates present in CE is improved and no additional chemical substances are introduced at the ionization stage. Therefore, possible interferences from the sheath solvents are eliminated. On the other hand, sheathless interfaces appear less stable due to deposits or flaking of the coating that cause a short lifetime. In addition, simultaneous adequate electrophoretic and ionization currents are not straightforward due to low liquid flows eluting from the CE capillary.

B. Ionization Sources

Analytes must be liberated from their associated solvent molecules as well as be ionized to allow mass separation. Several ionization methods enable ion production from the condensed phase and have been used for the coupling of CE to MS. Among them, atmospheric pressure ionization (API) methods, matrix-assisted laser desorption/ionization (MALDI), and inductively coupled plasma (ICP) ionization are mainly used. API techniques are undoubtedly the most widespread ionization sources and cover different analyte polarity ranges.

I. Atmospheric Pressure Ionization Sources

(a) Electrospray Ionization:

Electrospray Ionization (ESI) is the predominant ionization method for on-line CE–MS as it is well suited for the analysis of ionizable or polar compounds ideally separated by capillary zone electrophoresis (CZE). ESI is a soft ionization method that produces, in the gaseous phase, ions from charged evaporating liquid droplets in a high electrical potential toward the MS. A countercurrent flow of heated gas is often used to speed up desolvation, and nebulization is assisted by a nebulizing gas surrounding the CE capillary. Advantages of ESI are its simplicity, high ionization efficiency in terms of ions released from charged droplets, and ability to produce multi-charged ions. The main limitation of CE–ESI/MS concerns the limited compatibility of electrophoretic buffers commonly used in CE–UV, such as phosphate or borate. Therefore, volatile electrolytes, such as formate, acetate, carbonate, and ammonium, are often recommended. Furthermore, BGE ionic strength must be low since the ionization process in ESI is impaired by highly conductive solutions. Selectivity modifiers such as micelles, microemulsions, ion-pairing agents, and chiral selectors are not suitable or can even be detrimental for on-line CE–ESI/MS since they may contaminate the MS ionization source and cause significant ion suppression. Different strategies have been reported to address these difficulties, such as the partial-filling technique (PFT) (see Section III.A.2) or capillary electrochromatography (CEC) (see Section III.D). To decrease the negative effect of BGE composition, other ionization sources such as atmospheric pressure chemical ionization (APCI) or atmospheric pressure photoionization (APPI) could be envisioned since both techniques vaporize the sample and the CE electrolyte prior to the ionization process.

(b) Atmospheric Pressure Chemical Ionization:

The basic principle of APCI consists in nebulizing the effluent that is transferred through a heated vaporizer allowing complete evaporation of the solvent. A “corona discharge” electrode is placed in the source next to the vaporizer and produces electrons initiating the
chemical ionization process with ionization of the gas formed from the evaporated effluent.\textsuperscript{72,73} Some attempts were reported for CE–APCI/MS hyphenation,\textsuperscript{74–77} but led to very poor sensitivities because most of APCI interfaces were initially designed for LC–MS. Since APCI is a mass-flow-dependent source, sensitivity is improved with LC flows but impaired with low flow rates conventionally encountered in CE.

(c) Atmospheric Pressure Photoionization:

APPI was introduced by Robb et al. in 2000\textsuperscript{78} as a complementary technique to ESI and APCI for broadening the range of ionizable analytes by API techniques.\textsuperscript{79,80} This ionization source is mostly used in the analysis of non-polar compounds.\textsuperscript{78,81–87} APPI source is very similar to APCI, i.e., the sample is vaporized in a heated nebulizer before ionization and non-volatile salts can be easily removed during this step. However, the ionization is afforded by a discharge lamp instead of the corona needle used in APCI. The absorption by the analyte of a photon, generally emitted from a krypton lamp, can occur if the lamp energy is higher than the analyte’s ionization potential (IP). To significantly improve the ionization process, a doping agent is often added\textsuperscript{86,87} to participate in the ionization process via a charge or proton-transfer mechanism to the compound of interest.\textsuperscript{88} For several reasons, implementing APPI instead of APCI can lead to an improvement in sensitivity (height-to-noise ratio, \(H/N\)). First, common solvents such as water, acetonitrile, or methanol are not affected by discharge lamp as their IP are above 10 eV\textsuperscript{89,90} thus resulting in a low background noise. Second, APPI sources appear to be less sensitive to ion suppression (compared to APCI and ESI).\textsuperscript{91–94} Finally, APPI achieves significantly better sensitivity than APCI over a wider range of flow rates, particularly at the very low flow rates generated by CE separations. Therefore, it can also be considered in the sheathless configuration.\textsuperscript{90,95} Currently, only few publications are dedicated to CE–APPI/MS\textsuperscript{96–100} with experiments achieved on a conventional CE–MS system equipped with the sheath-flow configuration. To adapt the APPI interface, a spacer is positioned between the nebulizer and the vaporizer, and the dopant is usually added within the sheath-liquid interface. A wider choice of CE electrolytes (buffer and additives) can be used for the analysis of both polar and non-polar compounds without background noise and source contamination. As indicated by Mol et al., sodium dodecyl sulfate (SDS) also has no negative effect on photoionization efficiency; therefore, APPI provides a good solution for MEKC–MS\textsuperscript{96} and for MEEKC–MS.\textsuperscript{100} As presented in Figure 2, analysis of five steroids was performed by MEEKC–APPI/MS without extensive background noise or contamination of the ionization source.

2. Matrix-Assisted Laser Desorption/Ionization Interfaces

In MALDI, the energy of a laser is used to vaporize the CE effluent and ionize the analyte molecules. A light-absorbing compound is needed as a matrix for energy absorption, and the solution used as the matrix can act as both CE electrolyte and laser energy absorber at the interface. The off-line hyphenation of CE with MALDI has been recently reviewed.\textsuperscript{101–105} It requires either direct sample deposition onto a MALDI target or CE fraction collection for subsequent MALDI/MS analysis.\textsuperscript{106–109} However, the complexity and the high cost of the equipment has prevented its widespread use, even though some successful applications are reported,\textsuperscript{110–113} particularly for the characterization of large molecules with molecular masses up to several hundred kilodaltons (kDa).\textsuperscript{114}

3. Inductively Coupled Plasma Ionization Interfaces

ICP interfaces are used to completely fragment the analyte of interest for elementary composition determination. The principal field of application for CE–ICP/MS is the analysis of metals for the selective detection of specific elements where low detection limits are
Applications of the technique in the field of trace-element speciation analysis are currently increasing with the strong interest in analysis of inorganic (metal ions determination), organometallic (mercury speciation), and bio-inorganic (selenium and arsenic speciation) compounds.

C. Analyzers

All mass analyzers can be potentially hyphenated to CE. However, the very high efficiencies achieved in CE lead to very short peak widths. Only few seconds are available to record the analyte signal; therefore, this short analysis time window should be compatible with the MS sampling rates. For example, full-scan acquisition over a large mass range with a single quadrupole can be problematic regarding both peak definition and sensitivity due to the relatively slow cycle time afforded by this analyzer (>1 s). Although most research on CE–MS was performed using single quadrupole instruments, combinations of CE with ion trap, triple-quadrupole, time-of-flight (TOF), and Fourier transform-ion cyclotron resonance (FT-ICR) mass spectrometers were also described. This section gives a short overview of the most common MS instruments hyphenated with CE. For additional information, the reader is referred to dedicated literature on MS.
Quadrupole mass spectrometers have been used mainly in CE–MS because they can be obtained at relatively low cost, possess small dimensions, and are easy to operate. As previously mentioned, the scanning process is relatively slow and allows operation of only a small fraction of the available ions, which is not suitable with very narrow CE peaks. The use of selected ion monitoring (SIM) mode greatly improves sensitivity and duty cycle, but it is not always appropriate for the detection of complex mixtures. This kind of analyzer is currently used as a low-resolution instrument and for quantitative determination.

Ion-trap mass spectrometers, also referred to as “quisters” (quadrupole ion storage traps), are three-dimensional analogues of the above-mentioned quadrupole systems. Ions of different masses are stored in the trap and released one at a time by scanning the applied voltages. Ion traps can accumulate ions of pre-selected $m/z$ values, with a resulting gain in sensitivity compared to single quadrupole systems. They also present faster scanning rates, to be able to record a large number of spectra per second. Therefore, ion traps make efficient use of incoming ions and offer the possibility of MS$^n$ experiments, providing additional information by multiple-stage fragmentation of the analytes.

The most notable advance has been the recent commercialization of TOF analyzers as detectors for CE. Basic principle of a TOF mass spectrometer involves measuring the flight time of an ion through the mass spectrometer, yielding its $m/z$ value. Fast acquisition rates that provide extremely short times to generate a mass spectrum are achieved by a TOF instrument allowing complete characterization of the CE peaks with the additional advantages of high mass resolution, high mass accuracy (<10 ppm), extended mass range, and high sensitivity (sub-femtomole detection limits). Therefore, new applications in the proteomics and metabolomics are accessible with CE–TOF/MS.$^{142,143}$

In terms of overall sensitivity (one molecule detection), mass resolution (in excess of $10^5$), mass accuracy (<2 ppm), scan speed, and MS$^n$ capabilities, FT-ICR analyzers remain a very promising technique for the on-line coupling with CE. However, its technical demands, in terms of vacuum technology, and its high price currently limits its use mostly to fundamental studies.

### III. APPLICATIONS

On-line coupling of CE with MS has generated a wealth of data in bioanalytical (“life science”),$^{23,142–151}$ pharmaceutical,$^{152–156}$ forensic,$^{157}$ and environmental$^{158–160}$ domains, and was the subject of a number of comprehensive reviews.$^{14,15,161}$ The reader is thus referred to these sources for a systematic coverage of the field and a more extensive discussion of CE–MS applications than can be given in this context. On the basis of this consideration, all applications of electromigration techniques coupled with MS are not intended to be covered. The present section is directed toward recent examples on the on-line coupling of CZE and related electrophoresis-based techniques with MS in the field of pharmaceutical research. Indeed, CE is mainly used in pharmaceutical industries for the determination of major drugs and their by-products in pharmaceutical preparations (quality assurance purposes) as well as active components and their metabolites in biological fluids.$^{162,163}$ Therefore, CE–MS emerges as a suitable technique that fulfills key requirements such as sensitivity, selectivity, and peak assignment certainty.$^{164}$

### A. CZE–MS

CZE is the most common separation technique in CE since high speed and high-resolution separations of low-molecular-weight acidic and basic compounds can be achieved
with the appropriate choice of BGE (see Chapter 2). Applications of CZE–MS for the
analysis of a broad range of pharmaceutical compounds have been extensively demon-
strated, and the present section gives a general overview of both achiral and chiral recent
applications.

I. Drug Analysis by CZE–MS

(a) Drug Discovery:
Physicochemical and pharmacokinetic profiling of new chemical entities allows the rapid
identification and elimination of compounds with unsuitable properties for further drug
development. For instance, the aqueous dissociation constant \( pK_a \) is an important
parameter as the ionization state of a drug affects its absorption, distribution, metabolism,
and excretion (ADME). CZE is an alternative method for \( pK_a \) measurement to commonly
used potentiometric titration or UV spectral shift methods, and the use of MS detectors
extends the application range to non-UV-absorbing and poorly soluble analytes. Moreover,
the additional selectivity offered by MS allows a greater pooling of compounds per analysis,
which provides an increased throughput particularly important in the drug discovery stage.
For instance, Wan et al. developed a method for the simultaneous \( pK_a \) measurement of more
than 50 compounds in less than 150 min with CZE hyphenated with an ion-trap mass
analyzer.

(b) Drug Impurity Profiling:
Impurity profiling of pharmaceutical products is another issue in the pharmaceutical
field. The speed and reliability of analytical data regarding impurities, which can be both
synthetic and degradative products, have a direct impact on the eventual success or failure of a
promising drug. In this context, CZE can advantageously be used as an orthogonal technique
to high-performance liquid chromatography (HPLC) since separation principles are
different. For instance, CZE–MS equipped with an ESI source and an ion trap was
implemented by Visky et al. in the method development approach to support impurity
profiling of galantamine formulations under harsh conditions. Two degradation products
were detected at concentrations lower than 0.05% and further identified by MS/MS as
shown in Figure 3.

(c) Drug Metabolism Study:
Biotransformation of a therapeutic agent to metabolites possessing different biological
and structural properties can modify both the extent and the duration of parent drug’s activity.
Therefore, the subsequent isolation and structural characterization of the resulting
metabolites are important to understand the pharmacological effects of therapeutic agents.
The high efficiency of CE, combined with the versatility of ionization techniques to analyze
metabolites of varying polarity and the power of MS/MS to provide reliable structural
information, evolves as an efficient tool in drug metabolism studies as well as a rapid and
sensitive screening strategy for drug candidate selection. Using CZE coupled to ion-trap
ESI–MS, the analysis of diphenhydramine metabolites in human urines was performed by
Baldacci et al., and CE–MS appeared suitable for identifying urinary drug metabolites for
which no standards were available.

(d) Illicit Drugs of Abuse:
Routine analysis of common designer drugs in human biological fluids (e.g., plasma,
urine) is a major concern in doping control, surveillance of drug substitution, clinical
toxicology, as well as forensic science. Method sensitivity is often an issue since many drugs
2. Chiral Drug Analysis by CZE–MS

An extremely important aspect in pharmaceutical research is the determination of drug optical purity.\textsuperscript{155,175} The most frequently applied technique for chiral separations in CZE remains the so-called dynamic mode where resolution of enantiomers is carried out by adding a chiral selector directly into the BGE for in situ formation of diastereomeric derivatives. Various additives, such as cyclodextrins (CD), chiral crown ethers, proteins, antibiotics, bile salts, chiral micelles, and ergot alkaloids, are reported as chiral selectors in the literature,\textsuperscript{176–178} but CDs are by far the selectors most widely used in chiral CE.
(a) Direct Coupling:
First experiments in chiral CZE–MS with CD were achieved by Sheppard et al.\textsuperscript{179} in 1995, who demonstrated selectivity and sensitivity advantages of MS for the separation of terbutaline and ephedrine enantiomers. An important gain in sensitivity (1000-fold) was obtained by MS compared to UV detection. Both the free drug enantiomers and the enantiomer–CD inclusion complexes were detected by MS, demonstrating, for the first time, the interference problem given by the presence of chiral selectors in the ionization chamber. Other authors such as Otsuka et al.\textsuperscript{180} recommended to work with non-aqueous conditions to reduce the noise afforded by the presence of heptakis(2,3,6-tri-O-methyl)-\(\beta\)-cyclodextrin (TM-\(\beta\)-CD) as chiral selector in the BGE. The negatively charged highly sulfated \(\gamma\)-CD (HS-\(\gamma\)-CD) was used by Iwata et al.\textsuperscript{181} for the simultaneous chiral separation of amphetamine-type stimulants. By using a relatively high concentration of HS-\(\gamma\)-CD, amphetamines migrated as negatively charged complexes and were detected at the anode (reversed polarity mode). The complexes were dissociated at the ESI interface set in cationic ionization mode, and only amphetamines were detected by MS. Unfortunately, the important electrophoretic current due to the presence of the negatively charged selector at high concentration led the authors to work in aqueous media without buffering constituents.

(b) Partial-Filling Technique:
Because interfacing chiral CE with ESI/MS was severely troubled by the presence of CD, the PFT was investigated. The PFT, first introduced by Valtcheva et al.\textsuperscript{182} and further modified by Tanaka et al.,\textsuperscript{183} involves the filling of a discrete portion of the CE capillary with BGE containing a chiral selector (i.e., partial filling). This method is particularly adapted in chiral CE–MS and presents major advantages over the traditional approach.\textsuperscript{184,185} Neutral CDs were initially used, but charged chiral selectors are currently preferentially employed.

(c) PFT with Neutral Chiral Selectors:
When using PFT with a neutral selector, it is quite difficult to avoid any entrance of the chiral selector into the ionization source, particularly at a high pH, where EOF is important. The use of BGE at low pH and/or coated capillary to minimize EOF is therefore mandatory. However, the coaxial sheath gas, which generally assists the ionization process, leads to an aspirating phenomenon of the chiral selector in the MS direction. Javerfalk et al.\textsuperscript{186} were the first to apply PFT with a neutral methyl-\(\beta\)-CD for the separation of racemic bupivacaine and ropivacaine with a polyacrylamide-coated capillary and an acidic pH buffer (pH 3). Cherkaoui et al. employed another neutral CD (HP-\(\beta\)-CD) with a PVA-coated capillary for the analysis of amphetamines and their derivatives.\textsuperscript{187} To prevent a detrimental aspiration effect, analyses were carried out without nebulization pressure. Numerous other studies presented excellent results such as the enantioselective separation of adrenoreceptor antagonist drugs using tandem mass spectrometry (MS/MS),\textsuperscript{188} the separation of clenbuterol enantiomers after solid-phase extraction (SPE) of plasma samples,\textsuperscript{189} or the use of CD dual system for the simultaneous chiral determination of amphetamine, methamphetamine, dimethamphetamine, and \(p\)-hydroxymethamphetamine in urine.\textsuperscript{190}

(d) PFT with Charged Chiral Selectors:
The use of a charged chiral selector is probably the best solution to improve the classical PFT when CE is hyphenated with MS. Better solubility, additional electrostatic interactions, and improvement of the stereoselective separation power afforded by the self-mobility of the chiral additives into the BGE are among the numerous advantages of these charged selectors. When electromigration of the chiral species and the analytes are opposite (PFT-countercurrent approach), the mobility difference between free and complexed analytes is increased, leading to a higher resolution than with a neutral chiral selector.\textsuperscript{191–194} In optimized countercurrent
conditions, analytes reach the detector while the charged chiral selector migrates toward the opposite side of the MS. Hence, the stability of the ionization is improved by minimal contamination of the source and detector during the electrophoretic run. Cationic and anionic chiral selectors were employed for the stereoselective CZE–MS analysis of acidic and basic analytes, respectively.

Vancomycin was one of the most employed chiral selectors for separating acidic compounds. Introduced by Armstrong et al. for the separation of a wide range of compounds, vancomycin is a glycopeptide antibiotic that contains numerous stereogenic centers and a high number of functional groups. Chiral anionic arylpropionic acids such as ibuprofen, etodolac, and their metabolites were analyzed by Fanali et al., and advantages of MS were shown by unambiguous compound identification for several unresolved peaks in biological matrices. Vancomycin and other charged antibiotics were also used by Tanaka et al. for the separation of isocitric acid lactone, ibuprofen, ketoprofen, warfarin, and camphor sulfonic acid.

Regarding basic compounds, which represent more than 85% of ionizable drugs in the pharmaceutical domain, numerous CZE–MS applications with negatively charged CD were developed. For instance, sulfobutylether-β-CD (SBE-β-CD) was used for the first time by Schulte et al. for the separation of pharmaceutical cationic analytes (etilefrine, mianserine, dimethindene, and chloropheniramine). The potential of the PFT-countercurrent in CZE–MS has been further demonstrated for the stereoselective analysis of other chiral drugs such as bupivacaine, mepivacaine, prilocaine and ketamine, fluoxetine, and methadone. The latter was also used as a model compound for a fundamental study on the PFT-countercurrent approach based on a chemometric approach. Analysis of complex samples such as biological matrices or plant material was also achieved, for instance, a drug metabolism study was performed as well, and a simultaneous enantioseparation of tramadol and its five phase I metabolites was accomplished by CZE–ESI/MS, using negatively charged CD combined with the PFT-countercurrent methodology. The same strategy was also applied to the chiral separation of amphetamine derivatives at very low levels (sub-nanogram per milliliter) using an electrokinetic injection. As depicted in Figure 4, concentration of 1 ppb of each analyte, corresponding to an enantiomeric concentration of 0.5 ppb, was detected.

It is noteworthy that the use of PFT-countercurrent requires the presence of these negatively charged chiral selectors at relatively low concentration inducing a low conductivity in the BGE. As a result, the generated current generally did not exceed the instrument limitation even when high voltage was applied, which led to high efficiencies.

B. NACE–MS

The recent introduction of non-aqueous media extends the applicability of CE. Different selectivity, enhanced efficiency, reduced analysis time, lower Joule heating, and better solubility or stability of some compounds in organic solvent than in water are the main reasons for the success of non-aqueous capillary electrophoresis (NACE). Several solvent properties must be considered in selecting the appropriate separation medium (see Chapter 2): dielectric constant, viscosity, dissociation constant, polarity, autoprotolysis constant, electrical conductivity, volatility, and solvation ability. Commonly used solvents in NACE separations include acetonitrile (ACN); short-chain alcohols such as methanol (MeOH), ethanol (EtOH), isopropanol (i-PrOH); amides [formamide (FA), N-methylformamide (NMF), N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMA)]; and dimethylsulfoxide (DMSO). Since NACE–UV may present a lack of sensitivity due to the strong UV absorbance of some solvents at low wavelengths (e.g., formamides), the on-line coupling of NACE
with MS is particularly interesting. In addition, low evaporation temperature and surface tension of organic solvents are additional features that are favorable for sensitivity and spray stability. Finally, the generated electric currents are lower in the presence of organic mixtures than in water, allowing stable CE–MS conditions, as illustrated for the analysis of six amphetamines in ACN/MeOH with formate buffer such as the BGE by NACE–ESI/MS.

Several reviews were published on NACE–MS, and numerous fundamental studies were performed to assess the influence of the solvent on CE and MS performance, either with a sheath liquid or a sheathless nanospray configuration. BGE with volatile electrolytes in MeOH, ACN, DMSO, FA, NMF, and DMF were evaluated for the separation of basic analytes with NACE–ESI/MS vs. CE–ESI/MS. Organic solvents improved the separation selectivity and sensitivity due to a better ionization from facilitated evaporation of the solvent. NACE–MS is also readily applied to impurity profiling. For example, Cherkaoui and Veuthey described a NACE–ESI/MS method for the simultaneous analysis of fluoxetine, its meta-isomer, and other related compounds. Under selected NACE conditions, baseline separation of the investigated compounds was possible in contrast to

**FIGURE 4** Chiral CE–ESI/MS analysis of five amphetamine derivatives and two pharmaceutical compounds. Total ion current (TIC) and extracted ion currents (XIC) of amphetamine (A), methamphetamine (MA), methylenedioxymethamphetamine (MDA), methylenedioxyamphetamine (MDMA), and methylenedioxymethylamphetamine (MDEA), and tramadol (TMD) and methadone (MTD) in plasma after LLE with electrokinetic injection.
aqueous CE where co-migration of the closely related compounds was observed and attributed to similar electrophoretic behavior causing insufficient resolution. Finally, the use of NACE-MS has proven to be valuable in pharmacological and clinical studies,\textsuperscript{1224,1225} as well as in investigations of drugs and metabolites.\textsuperscript{12,226–230}

\section*{C. MEKC-MS}

Besides CZE and NACE, micellar electrokinetic chromatography (MEKC) is also widely used, and ionic micelles are used as a pseudo-stationary phase. MEKC can therefore separate both ionic and neutral species (see Chapter 2). Hyphenating MEKC with ESI/MS is problematic due to the non-volatility of micelles, which contaminate the ionization source and the MS detector, resulting in increased baseline noise and reduced sensitivity. However, MEKC-ESI/MS was applied by Mol et al. for identifying drug impurities in galantamine samples.\textsuperscript{231,232} Despite the presence of non-volatile SDS, all impurities were detected with submicrogram per milliliter sensitivity and could be further characterized by MS/MS.

To overcome the limitations of MEKC-MS, several methods have been implemented: (i) the use of volatile surfactants,\textsuperscript{233} (ii) the use of low-molecular-weight (unpolymerized) surfactants either at low concentrations\textsuperscript{70} or with the previously described partial-filling approach (see Section III.A.2),\textsuperscript{234–240} (iii) the use of high-molecular-weight surfactant, and (iv) the use of APCI or APPI sources instead of ESI.\textsuperscript{241} The possibility of using high-molecular-weight surfactants is an attractive approach because these polymerized micelles are difficult to ionize due to the covalent bond formed between the surfactant monomers. Moreover, they exhibit a low surface activity resulting in a stable spray and a signal increase in the MS.\textsuperscript{242} Because APCI and APPI processes are less affected by non-volatile salts, they also provide several possibilities for MEKC-MS to reduce background noise and source contamination. Takada et al. reported an MEKC-APCI/MS method for xanthine derivatives using SDS directly introduced into the ionization source of the MS detector without a severe decrease in sensitivity.\textsuperscript{243} Mol et al. demonstrated that SDS had also little negative effect on photoionization efficiency and MEKC-APPI/MS was successfully applied for both polar and non-polar compounds.\textsuperscript{96} The analysis of enantiomers by chiral MEKC-MS is also readily performed, and Shamsi was the first to report the use of chiral surfactants.\textsuperscript{244} However, most chiral surfactants substantially contribute to chemical noise and suppress analyte ionization because of micelle dissociation during ionization process into surfactant monomers with high surface activity.

\section*{D. CEC-MS}

CEC (see Chapter 17) is an analytical approach that combines the advantages of both electrophoresis and partitioning separation processes, i.e., the high efficiency of CE with the high loading capability of particle-based columns.\textsuperscript{245} In CEC, capillary columns are usually classified into three main formats: (i) packed-CEC, where a fused-silica capillary is filled with a typical HPLC packing material; (ii) open-tubular CEC (OT-CEC), where the retentive stationary phase is present only on the walls of the capillary;\textsuperscript{246,247} and (iii) monolithic-CEC, where a monolithic stationary phase is prepared by in situ polymerization within the capillary. The main drawback of packed-CEC is the production of frits and the need for junctions between transfer segments and the filled capillary to prevent packed bed movements under EOF. The pressurized inlet and outlet reservoirs are also used in most CEC systems to suppress bubble formation associated with frits.\textsuperscript{248} Continuous bed-type columns such as OT-CEC and monolithic-CEC columns have thus emerged as alternatives to packed-CEC to circumvent
these problems and obtain a completely frit-less packed column system that eliminates band broadening, peak distortion, or bubble formation.249

The three types of support are used in CEC−MS coupling, and hyphenation appears effortless since additional selectivities (e.g., hydrophobic or chiral interactions) are given by the stationary phase. Therefore, CEC can overcome some drawbacks of CE−MS coupling and prevent source contamination by non-volatile additives such as CD or SDS. The first interface ever used for the hyphenation of CEC with MS was the continuous flow-fast atom bombardment (CF-FAB) interface.250,251 Because of technical difficulties to maintain a stable electrical current, this type of interface was replaced by the more convenient API interfaces. Few reports exist on the hyphenation of CEC with MS, using APCI252 or APPI,99 and main developments involve ESI coupling. Considerations encountered in interfacing CEC−MS with API sources are analogous to those met in CE−MS, i.e., electrical contact and flow rate issues.253 Similar to CE−MS coupling (See Section II.A), both can be overcome with an appropriate interface such as the sheath-flow or the sheathless configurations.254 Whereas OT-CEC and monolithic-CEC do not require a special setup, care must be paid when coupling packed-CEC with MS. As mentioned earlier, the use of pressurized inlet and outlet reservoirs is recommended to suppress bubble formation and such pressurized system are currently not compatible with API sources. Therefore, packed columns are directly connected to the interface, and three different configurations of column outlet were reported. The first configuration uses columns with fritted termination255−258 and consists in using only the packed portion of the capillary column, i.e., without open connecting tube. Therefore, the column is terminated after the outlet retaining frit and the CEC effluent is directly sprayed into the atmospheric area of the ion source. This configuration is generally used with a sheath-flow interface. The next possibility is using columns with tapered ends as flow restrictors where the packed capillary column can be either externally259 or internally133 tapered. Both configurations are preferentially used in a sheathless arrangement, and CEC columns with an integrated, conductive nanospray tip have been recently commercialized. The third employs columns with a connecting tubing and uses a piece of fused-silica capillary as the connecting device, generally in a coaxial sheath-flow or liquid-junction configuration.260,261 In this arrangement, the CEC column is electrically grounded after the terminating frit and the connecting tube is coupled to the packed-CEC column via a zero-dead-volume union. This configuration presents the advantage that backpressure due to the flow through the open tube is enough to overcome the frits issue. Furthermore, tip dimensions are usually decreased, resulting in a very stable spray. Applications of CEC, including the determination of pharmaceutical compounds and impurities, as well as chiral separations, were extensively reported.262,263 The next section gives a brief outline of some relevant applications.

I. Drug Analysis by CEC−MS

CEC−MS was applied for the analysis of various pharmaceuticals using either packed-CEC,264−269 OT-CEC,270,271 or monolithic-CEC columns.

(a) Packed-CEC−MS:

Lord et al. analyzed a mixture of steroids by CEC−ESI/MS and interfaced externally tapered CEC columns in both sheathless and sheath-flow arrangement. Sensitivity was found 20-fold higher in the sheathless configuration.259 The same conclusion was drawn by Warriner et al., who evaluated CEC-nanospray/MS vs. CEC-microspray/MS with an ion trap using five corticosteroids.272 Cahours et al. used CEC−ESI/MS for a drug metabolism study and obtained a simultaneous baseline separation of flunitrazepam and its major metabolites. For CEC−ESI/MS coupling, the commercially available packed-CEC column was connected
without any dispersive effect using a Teflon connection. CEC–ESI/MS was also implemented in high-throughput drug discovery by Paterson et al. who reported the separation of 13 structurally related compounds from a parent drug candidate. Finally, electroosmotically driven solvent gradients were used for the separation of 12 amino acids by reversed-phase CEC coupled with TOF/MS. Capillary columns with an internal taper at the column outlet were used in the sheath-flow configuration.

(b) OT-CEC–MS:
Zhu et al. coupled OT-CEC to ESI/MS for the analysis of β-blockers and benzodiazepines. The authors described the use of a polymeric surfactant as a stationary-phase coating that enabled minimal surfactant introduction in the MS compared to MEKC–ESI/MS, thus avoiding interferences from non-volatile micelles in ESI/MS.

(c) Monolithic-CEC–MS:
Kato et al. evaluated three different silica monolithic columns by changing the poly(ethylene glycol) (PEG) contents for the simultaneous analysis of cationic, neutral, and anionic compounds using CEC–ESI/MS with a sheath-liquid configuration. Que and co-workers also developed novel types of polar monolithic-CEC columns for the analysis of complex mixtures of saccharides with CEC–ion-trap MS and CEC–FT-ICR MS, enabling low-femtomole sensitivities with mass accuracy <4 ppm in the m/z range of 200–2000. This column technology provided a nearly universal system that could separate a wide range of carbohydrates, including monosaccharides and oligosaccharides, saccharide alditols, and anomers.

2. Chiral Drug Analysis by CEC–MS

As discussed in previous sections, adding a chiral selector to CZE (see Section III.A.2) or MEKC (see Section III.C) buffers, either directly or indirectly using PFT, is possible for analysis of CE–ESI/MS enantiomers. However, the use of such chiral selectors or additives can produce a significant enhancement of background noise. An alternative is to attach or bond the chiral selector as a chiral stationary phase (CSP) either to a packed-CEC or monolithic-CEC column, or to an OT-CEC column.

(a) Chiral Packed-CEC–MS:
Brush-type, proteins, CDs, natural molecular imprint-based polymers (MIP), and macrocyclic antibiotics have been immobilized as chiral selectors on packed-CEC columns. Zheng and Shamsi demonstrated the possibility of using chiral CEC–ESI/MS with a commercially packed column for the determination of warfarin enantiomers in human plasma using coumachlor as an internal standard (IS). Robustness of this chiral CEC capillary was recently improved by a novel procedure and applied for the simultaneous enantioseparation of height β-blockers with multimodal CSP using different combinations of vancomycin and teicoplanin, as presented in Figure 5.

(b) Chiral OT-CEC–MS:
Although the majority of chiral CEC–MS applications still involve packed columns, few reports on chiral OT-CEC–MS are found in recent literature. The feasibility of coupling OT-CEC (using a short Chirasil-Dex-coated capillary column) to MS and MS/MS for trace analysis of hexobarbital enantiomers in biological fluids was reported by Schurig and Mayer. More recently, Kamande et al. investigated polyelectrolyte multilayer (PEM) coating as a new medium for the separation of chiral analytes, and PEM-coated capillaries were successfully coupled to ESI/MS for the stereoselective analysis of five β-blockers.
IV. QUANTITATIVE ASPECTS

Quantitative CE–MS studies were scarcely reported.126,173,291–293 This subject is however of prime importance, particularly for the pharmaceutical industry where the reliability of analytical data is essential. For this reason, method development is generally followed by an evaluation of quantitative performance using an appropriate validation procedure performed in agreement with criteria established by the International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH) and the Food and Drug Administration (FDA) guidelines, or Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) commissions.294–298

A major difficulty in terms of quantitative CE–MS studies can be linked to the sheath-flow interface geometry. Instrumental measurements of ionization currents were described by Bruins66 and depend on different parameters, including the positioning of the capillary inside the nebulizer. An incorrect or unrepeatable positioning of the capillary inside the interface leads to an unstable spray, which in turn yields inaccurate ionization performance and low sensitivity. Therefore, tests must be performed prior to starting a sequence of analyses to adjust the capillary position and ensure that these electric currents remain stable. Geiser et al. suggested a daily procedure based on constant ionization currents to achieve good sensitivity and enhanced system stability. With this applied procedure, precision and trueness were considerably improved.299 A second possible source of error is generally attributed to the electrophoretic process itself and to the impossibility to obtain uniform EOF, particularly in presence of capillary laminar flow due to both the nebulizing gas and the sheath liquid. These effects also appear to be detrimental for repeatable sample injections. Finally, it is difficult to correctly thermostate the final part of the capillary in CE–MS configuration, and this can lead to shifts in migration times and/or peak broadening.

To eliminate problems associated with quantitation in CE–MS and reduce the impact of system variability on method accuracy, it is mandatory to use an IS. The variability obtained in CE–MS, typically around 20%, can be lowered significantly when the IS is taken into account. Using a simple structurally unrelated IS, the precision of a CE–MS method can be improved by a factor of 2300 and increased up to a factor 4 with a closely migrating
compound. However, even with closely migrating IS, analyte and IS still reach the ionization source at different times and short-term variations of the ionization process may be of concern, particularly in ESI. To achieve repeatable results, ionization conditions must be constant, without ion suppression or enhancement, namely matrix effects. Matrix effects have been described by several authors, particularly for LC–ESI/MS analysis of biological samples. Evaluation of the matrix effect usually consists of a post-column infusion of the analyte mixture while different matrices are injected onto the chromatographic system, leading to the visualization of ionization suppression or enhancement. Adapted from the configuration proposed by Bonfiglio et al., a post-capillary infusion system was used with the sheath-liquid interface as an analyte-delivering device. A drug mixture solution was continuously infused with the sheath liquid through the nebulizer and effects associated to the migration of interfering compounds were assessed by measuring MS responses with the injection of blank or spiked matrices.

Ionization changes can be efficiently corrected with the use of an isotopically labeled IS, which possesses identical ionization response and fragmentation pattern. Therefore, deuterated IS can be used to correct both the overall method variability (e.g., sample preparation, injection, electrophoretic process, etc.) as well as matrix effects since the amount of suppression from interferents is expected to be similar. However, the total concentration of analyte and IS should be below the saturation of the ionization process. Guidelines to obtain a reproducible CE–MS method were published by Ohnesorge et al. and took into account the use of an isotopically labeled IS.

Because most of the uncertainty and potential non-linearity in MS do not refer to the analyzer but rather to the ionization process, all types of analyzers can be used for quantitation. Sensitivity, scan speed, mass resolution, and cost are the key points to be compromised. In the case of target analysis, the sensitivity, and thus accuracy, is best for quadrupole instruments. Generally, the time resolution should be adapted to the small peak widths of CE; therefore, the SIM mode is preferred, which also improves sensitivity. Multitarget analysis and screening can be better performed with ion trap or TOF mass spectrometers. On the other hand, the use of MS/MS can greatly enhance selectivity using the selected reaction-monitoring (SRM) mode that ensures more accurate measurements and lower limits of quantitation (LOQ) by reducing the chemical noise.

Finally, when comparing precision, number of samples, analyte concentration, and ionization yield should be considered. A small amount of data can lead to unrepresentative values. Furthermore, when the concentration is close to the LOQ, the variability increases and analytes with higher ionization efficiencies yield present lower relative standard deviations (RSD) values because of $H/N$ differences.

V. NEW DEVICES

Chip-based microdevices represent a new emerging and particularly advanced technology from the early work of Manz and co-workers in 1990. The technology relies on the integration of different steps of the analytical process into a miniaturized flow system. Potential microchip’s major advantages are: high speed for maximum “time-to-result decrease,” reduced sample volume and reagent consumption, integration of operational elements, disposability, portability, and high-throughput capabilities via parallel processing or automation. They are engineered for sample preparation such as SPE; solid-phase microextraction (SPME); LLE; protein digestion; sample desalting; or derivatization, preconcentration, and for different separation strategies, namely CE, CEC, CEC, and nano-HPLC. However, the latter technique is less popular since electrokinetically driven flows are more easily integrated to microstructures. It is simpler...
to establish a voltage drop across microchannels than pressure drops that would require miniaturized valves and pumps. Furthermore, microchips often include integrated detection methods such as optical and EC detectors, and can also be hyphenated to MS. Although there are many efforts invested in developing miniaturized and multiplexed MS, the main drawback of this coupling presently is that MS cannot be miniaturized and integrated to the microchip. Early microfluidic systems hyphenated with MS were mainly used as sample delivery devices for infusion experiments, with the potential to rapidly load a large number of different samples. Recently, new developments enable the combination of MS with complex microdevices that integrate the whole analytical process. The present section is restricted to the description of microfluidic separation devices hyphenated with MS in which the flow is electrokinetically driven. It will focus on fabrication methods, microchip designs, MS interfacing, and applications.

A. Fabrication Methods

CE chips are mainly obtained using various glass substrates, from inexpensive soda-lime glass to high-quality quartz. Various polymer materials are also used. The choice of a particular material depends on its surface properties, ease of fabrication, which can be quite different according to the material origin, disposability, and price. Microfabrication processes were recently reviewed and the reader is thus referred to dedicated literature for additional useful information on microfluidic device fabrication.

1. Silica-Based Microfabricated Devices

Glass substrates are most commonly used because of their good optical properties, well-understood surface characteristics, and well-developed microfabrication methods adapted from the microelectronics industry. Structures on glass substrates are usually generated using standard photolithographic technologies. The glass substrate is coated with a sacrificial and a photoresist layer, which is exposed to UV light in a region defined by a photomask presenting the appropriate pattern design. The mask pattern is then chemically etched with hydrofluoric acid. Following microchannel etching, the photoresist and sacrificing mask layers are removed. Finally, the substrate is bonded to another piece of substrate to form a finished microchip. Thermal diffusion is the most often used method for glass bonding as well as chemical-activated bonding and adhesive annealing.

2. Polymer-Based Microfabricated Devices

Optical properties of the material are less critical for microchips hyphenated with MS than for devices with on-chip optical detection where low background absorption or fluorescence is mandatory. Thus, completely opaque polymers like glassy carbon or polyimide can be used as microfabrication substrates. Furthermore, polymer microchips are of great interest because their potentially low manufacturing costs may allow them to be disposable. Methods used for the fabrication of plastic chips include laser ablation and molding methods.

The photoablation process consists of the absorption of a short-wavelength laser pulse to break covalent bonds in polymer molecules and eject decomposed polymer fragments. Channels of various geometries and dimensions can be obtained using an appropriate mask. Many commercially available polymers can be photoablated, including polycarbonate, poly(methyl methacrylate) (PMMA), polystyrene, nitrocellulose, poly(ethylene terphtalate) (PET), and poly(tetrafluoroethylene) (Teflon).
The formation of microchannels using molding methods involves the fabrication of a molder, followed by the transfer of the channel pattern from the molder to the polymeric substrate, and finally microchannels’ enclosure. In contrast to chemical etching (i.e., isotropic etching), microchannels obtained with molding methods possess vertical walls. Depending on the channel dimensions and precision requirements, the molder can be produced with various techniques. For instance, photolithography of an X-ray resist was performed for very small channels (< 100 μm), followed by electroplating to form the suitable molder. Replication of the molder to produce microchips can be accomplished by injection molding, embossing, or casting. In the injection molding process, the polymer is melted and injected against the molder in a molding chamber. In the embossing process, the polymer substrate and the embossing tool are heated separately under vacuum to a temperature just above the glass transition temperature of the polymer material, and then they are brought into contact. The casting process involves polymer material that is poured onto the top of the molder and hardened at atmospheric pressure and temperature. Finally, the grooves are sealed with thermal lamination to form microchannels. Thus, plastic chips possess two different surface types, three walls of the polymer substrate, and one wall of the laminated film. Special care must be given to well match the materials to avoid disrupted plug flows. An alternative consists in annealing the molded plate directly to another plate with oxygen plasma. With this technique, channels with four equivalent walls are formed and separation is less impaired.

B. Designs

A typical CE microchip is around 10 cm² in size and a few millimeters thick. Dimensions of the microchannels are 1–5 cm in length, 5–50 μm in depth, and 20–100 μm in width, although channels with depths of less than 5 μm were reported. Thus, the small cross section of the channels allows Joule heat dissipation, thus high electric fields can be applied on microchips to achieve sub-second separations. The change of the classic CE format with relatively long effective separation length to shorter microfabricated channels on microchips enables rapid separations without significant peak broadening. Because efficiency is independent of the migration length, shorter separation paths can be used without loss in resolution. However, short separation channels on microchips make the sample introduction more critical. Apart from usual stacking procedures, separation channel’s length can be increased by introducing serpentine- or spiral-shaped channels without any increase of zone dispersion from turns.

The design of microchips for CE has undergone significant development from single-channel structures to increasingly complex models, and current designs allow on-chip reactions and separation in multiple channels. Arrays of separation channels were also designed on microchips to simultaneously analyze a large number of samples, but they are usually constrained by the detection method. A basic microchip includes two crossed channels and four reservoirs for sample, waste, and cathode and anode electrolytes. As mentioned above, the controlled injection of nanoliter or picoliter sample volumes in the separation channel is required to minimize dispersion effects. Electrokinetic injections are the most widely used sampling techniques since no additional elements such as valves or pumps need to be integrated onto the microstructure. Many injection schemes have been developed and the integrated injectors are usually either cross-channel injectors, formed by orthogonally intersecting the sample-to-waste channels, or double-T injectors, where the two arms of the sample-to-waste channels are offset to form a larger injector region. To date, cross-intersection is mostly employed, and with this design, several injection techniques such as floating, pinched, dynamic, and gated injections can be implemented depending on electric field strength distributions.
Implementation of microanalytical devices presents some issues mostly related to the scale of the volumes. In fact, successive reduction in the sample volume may compromise analysis either because the measurement limit of the analytical method is exceeded or because the sample is no longer representative of the bulk specimen. Another drawback for microchip devices is microvolume evaporation of both sample and reagent from the microchip, compromising quantitative determination or inducing unwanted hydrodynamic flows. This problem has been addressed by designing pipetting systems that automatically replace fluid lost by evaporation or by enclosing the chip in a controlled environment.379

C. MS Interfacing

Over the past decade, significant progress in the field of microfluidics, instrumentation miniaturization, and the integration of the whole analytical process has evolved, but microchip–ESI/MS interfacing has also received a great attention since it combines small sample volume’s handling, fast separation with sensitive, nearly universal detection, and possible structure elucidation of separated compounds. Furthermore, despite the mismatch in physical dimensions, MS is a promising alternative to optical and EC detections due to the perfect and natural compatibility between flow rates required for ESI/MS and those generated by CE chips. An overview on the combination of microsystems and MS was given in general reviews,380–382 and three approaches are currently distinguished to interface microfluidic devices to MS: (i) spraying directly from the chip, (ii) spraying from a capillary sprayer attached to the chip, or (iii) using a nanospray emitter integrated into the microchip. Several designs are illustrated schematically in Figure 6.

I. Spraying Directly from the Chip

The first on-chip separations prior to MS were reported in 1999 by several groups and consisted in spraying the fluid out of the microchip directly from the microchannel.324,383 The main focus was to create stable ESI conditions and reduce background noise. Designs were quite attractive because they did not require any complex setup, since the outlet was simply formed by dicing the chips. On the other hand, the flat-face from which the sample was sprayed led to the formation of large droplets at the surface of the chip, which caused excessive band broadening and sample dilution. Attempts were made at minimizing droplet size by coating the outlet orifice with a hydrophobic agent or by pneumatically assisting the droplet formation.384 However, these approaches did not provide efficiencies as high as obtained with a transfer capillary or a nanospray emitter. Another methodology was implemented by Girault and co-workers,349,385 who developed a polyimide microfluidic system by plasma etching with a precise nanospray exit nozzle designed in a tip shape. The typical isotropic etching of the plasma as well as the thickness of the tip edge (only 20 μm) contributed to the onset of a spray in contrast to thicker devices in which droplets are formed at the outlet. Furthermore, the thin outlet walls made of hydrophobic polymer avoided the wetting of the spray edge, resulting in the formation of an efficient and stable nanospray.

2. Spraying from a Capillary Sprayer Attached to the Chip

Although previous studies showed that direct ionization from the outlet separation channel at the chip surface with Taylor cone formation could be possible, this is probably not optimal because the ESI cone volumes are larger than peak volumes. With short
transfer capillaries attached to the microdevice, as first introduced by Figeys et al., an external electrospray is implemented. Special care must be paid when using transfer capillaries for joining microfluidic devices. Precise, low dead-volume alignment of the chip separation channel and transfer capillary is mandatory for maintaining efficiency, especially because injected sample volumes on the microchips range from 0.1 to 0.5 nL compared with 2–5 nL in conventional CE. Thus, Bings et al. developed a method to obtain low dead-volume connectors, as small as 0.7 nL. When the spray is generated from a transfer capillary, either a disposable nanospray emitter or a fused-silica capillary transfer line can be inserted in the microchip. For the former, the ESI nozzle is usually made with a sharp, tapered capillary end from which the liquid sprays to produce small, well-defined droplets. An advantage of this setup is that the nanospray tip can be removed and replaced without changing the microchip, as clogging is a significant problem when using capillaries with tapered ends. For the latter, interfacing options are similar to those used in conventional CE–MS coupling and include liquid junction and coaxial sheath-flow configurations.

**FIGURE 6** Schematic representation of different interfaces for chip CE–ESI/MS: (A) spray directly from the chip, (B) liquid-junction capillary interface, (C) gold-coated capillary interface, and (D) coaxial sheath-flow configuration. Reprinted from reference 410 with permission from Elsevier Science B.V.
3. Spraying from an Integrated Nanospray Emitter

Microchips’ fabrication with integrated tips can result in improved spray repeatability and efficiency since alignment and dead volume are not a critical issue anymore. However, production of fine and robust nanospray emitters as an integral part of a microdevice is not trivial, and highly specialized microfabrication procedures are required. Microfluidic devices with integrated ESI tips have been produced for infusion experiments, but to date, no microchips with such a design was fabricated for CE separation prior to MS detection.

D. Applications

The most prominent field of applications for microchip–MS concerns identification and analysis of large molecules in the field of proteomics according to the reduced separation time compared to conventional approaches such as gel-based methods for protein analysis. High-throughput analyses, with lower contamination and disposability, are other features of microfabricated devices that allow the fast screening of proteomic samples in the clinical field. Applications also include the analysis of low-molecular-weight compounds such as peptides or pharmaceutical samples.

Henion and co-workers investigated the use of microfabricated devices coupled with MS for the determination of several drugs. In a first study, they evaluated the potential of a polymer-based microchip CE–MS system for on-chip separation and quantitative detection of polar small molecules. A silicon master was microfabricated using photolithographic and dry etching processes; microchannels were embossed in the plastic and thermally enclosed with the same polymer. The microchip was coupled to a microsprayer via a liquid junction formed between the chip and sprayer, and a triple-quadrupole mass spectrometer was operated in SIM and SRM modes to produce CE–MS results. No surface treatment of the polymer was necessary to obtain a sufficient and repeatable EOF. A baseline separation of carnitine, acylcarnitine, and butylcarnitine was obtained in less than 10 s for injection quantities of 0.2 nmol of each compound. This group assessed the applicability of another glass device for quantitative microchip CE–MS analysis of the above-mentioned drugs in human plasma and urine samples. LOQ as low as 5 μg/mL were obtained with good performance in terms of trueness and precision, which demonstrated the feasibility for on-chip CE separation and ESI/MS detection in bioanalytical applications. Tachibana et al. described a robust and simple interface for microchip CE–MS using a spray nozzle connected to the exit of the separation channel of the microchip. The analysis of several basic drugs such as pindolol, nicardipine, sulpiride, and trimipramine was successfully performed in less than 20 s using the optimized system that consisted of a spray nozzle with a small bore size and a separation buffer with high viscosity. This system was also applied to the separation of peptides and trypsin-digested proteins with the adsorption of peptides to the quartz microchip alleviated by adding acetonitrile to the separation buffer.

VI. SUMMARY AND CONCLUSIONS

The use of different electromigration-based separation techniques hyphenated with MS has become a standard technique in modern pharmaceutical analysis. With the possibility of several commercially available instruments, including various interfaces, the coupling between CE and MS can now be easily achieved. Therefore, numerous issues can be resolved according to the wide choice of operation techniques afforded in CE and the various ionization modes and/or analyzers. CE–MS has emerged as a good alternative for trace
analysis (i.e., degradation substances, impurities) or for compounds without strong chromophores such as sugar, peptides, or amino acids. Furthermore, MS detection in the SIM mode enhances selectivity and sensitivity, and expands the potential for quantitation using CE. Despite the fact that CE–MS is widely used in qualitative analyses, few quantitative applications have been published for biological matrices and pharmaceutical formulation analyses.

Much progress has been made in coupling microfabricated devices with MS, and this field continues to move forward with the commercialization of new microfluidic systems. The latter allow the handling of very small samples with short analysis times and at reduced analysis costs. Furthermore, microfabrication enables large-scale integration of the entire analytical process, multiplexing, and therefore high-throughput analysis. Thereby, this emerging technology may become a dominant force in the very near future, as it perfectly couples the concept of limited sample amounts with the requirements for high separation efficiency and detection sensitivity. An important requirement for high-throughput qualitative and quantitative determination of small molecules in pharmaceutical drug discovery also demands highly selective screening methods for further testing. To achieve this goal, new developments such as multiplexed chip–MS are promising.

**ABBREVIATIONS**

<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>ACN</td>
<td>Acetonitrile</td>
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<tr>
<td>APCI</td>
<td>Atmospheric pressure chemical ionization</td>
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<td>API</td>
<td>Atmospheric pressure ionization</td>
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<td>APPI</td>
<td>Atmospheric pressure photoionization</td>
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<td>BGE</td>
<td>Background electrolyte</td>
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<td>CD</td>
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<tr>
<td>CE</td>
<td>Capillary electrophoresis</td>
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<tr>
<td>CEC</td>
<td>Capillary electrochromatography</td>
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<tr>
<td>CF-FAB</td>
<td>Continuous flow-fast atom bombardment</td>
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<tr>
<td>CSP</td>
<td>Chiral stationary phase</td>
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<tr>
<td>CZE</td>
<td>Capillary zone electrophoresis</td>
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<tr>
<td>DMA</td>
<td>N,N-dimethylacetamide</td>
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<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
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<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
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<tr>
<td>EC</td>
<td>Electrochemical</td>
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<td>EOF</td>
<td>Electroosmotic flow</td>
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<td>ESI</td>
<td>Electrospray ionization</td>
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<tr>
<td>EtOH</td>
<td>Ethanol</td>
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<td>FA</td>
<td>Formamide</td>
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<td>FDA</td>
<td>Food and drug administration</td>
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<tr>
<td>FT-ICR</td>
<td>Fourier transform-ion cyclotron resonance</td>
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<tr>
<td>H/N</td>
<td>Height-to-noise ratio</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
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<tr>
<td>HP-β-CD</td>
<td>Hydroxypropyl-β-cyclodextrin</td>
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<tr>
<td>HS-γ-CD</td>
<td>Highly sulfated γ-cyclodextrin</td>
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<tr>
<td>ICH</td>
<td>International conference on Harmonisation</td>
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<tr>
<td>ICP</td>
<td>Inductively coupled plasma ionization</td>
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<tr>
<td>IP</td>
<td>Ionization potential</td>
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<td>i-PrOH</td>
<td>Isopropanol</td>
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<td>IS</td>
<td>Internal standard</td>
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</table>
LC  Liquid chromatography
LIF  Laser-induced fluorescence
LLE  Liquid–liquid extraction
LOQ  Limit of quantitation
m/z  Mass-to-charge ratio
MALDI Matrix-assisted laser desorption/ionization
MEEKC Microemulsion electrokinetic chromatography
MEKC Micellar electrokinetic chromatography
MeOH Methanol
MIP Molecular imprint-based polymer
MS  Mass spectrometry
NACE Non-aqueous capillary electrophoresis
NMF N-methylformamide
OT-CEC Open-tubular capillary electrochromatography
PEG Polyethylene glycol
PEM Polyelectrolyte multilayer
PET Polyethylene terptalate
PFT Partial-filling technique
PMMA Polymethylmethacrylate
RSD Relative standard deviation
SBE-β-CD Sulfobutylether-β-cyclodextrin
SDS Sodium dodecyl sulfate
SFSTP Société française des sciences et techniques pharmaceutiques
SIM Selected ion monitoring
SPE Solid-phase extraction
SPME Solid-phase microextraction
SRM Selected reaction monitoring
TIC Total ion current
TOF Time-of-flight
TM-β-CD Heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin
XIC Extracted ion current

REFERENCES


