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Review

Review coupling of capillary electrochromatography to mass spectrometry

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Abstract

This review discusses the development of capillary electrochromatography (CEC) coupled to mass spectrometric (MS) detection over the last few years. Major topics addressed are instrumental setups employed and applications of this technology published in the recent literature. The instrumental section includes a discussion of the most commonly used interfaces for the hyphenation of CEC and MS as well as ionization techniques. Applications reviewed in this paper come from a variety of different fields such as the analysis of biomolecules like proteins, peptides, amino acids or carbohydrates, chiral separations or the analysis of pharmaceutical an their metabolites in a series of matrices.
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Keywords: Reviews; Electrochromatography; Mass spectrometry; Interfaces; CEC–MS

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1. Introduction

Within the last two decades capillary separation techniques have gained increased importance, as can be seen from the large number of original papers, reviews and books published within this field [1–6]. Focusing on the dominating separation principle involved, chromatographic techniques like capillary high-performance liquid chromatography (cHPLC) [7], electrophoretic techniques, e.g. capillary zone electrophoresis (CZE) [8] and hybrid techniques can be distinguished. The latter include methods employing so-called pseudo stationary phases like micellar electrokinetic chromatography (MEKC) and the more recently developed microemulsion electrokinetic chromatography (MEEKC) [9,10] and techniques where true stationary phases are used like capillary electrochromatography (CEC) [4,11]. A major benefit of these hybrid techniques is that due to the combination of two almost complementary separation principles, namely chromatographic and electrophoretic mechanisms,

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unique selectivities can be achieved. Strictly speaking, the possibility of extensive selectivity tuning by changing the properties of the stationary phase is more or less restricted to CEC, were the full range of packing materials developed for HPLC can be applied. An additional outstanding attribute of CEC is, that impressive efficiencies with plate numbers above 10^6/m have been obtained employing this separation method [12]. Whereas the column performance can be improved by reduction of column diameters down to the capillary format, this development can cause a series of problems with detection, especially when the commonly used spectrophotometric detection techniques are considered. Particularly in the case of on-capillary detection the short optical path length results in reduced sensitivity with respect to the analyte concentration. To overcome this problem a substantial effort has been made to combine these capillary separation techniques with more suitable detection methods, like mass spectrometric (MS) detection, where sensitivity does not depend on the size of detection cells.

Another reason for combining MS with separation methods is the increase in information provided by MS spectra which allows a higher degree of certainty in peak assignment than simple comparison of UV spectra. When it comes to the characterization of unknown compounds, MS spectra and even more multiple MS (MS^n) experiments can supply additional useful hints, leading to the elucidation of their chemical structure. Finally, discussing the analysis of samples with complex matrices, MS detection often enables the extraction of analyte signals even from a very high level of background noise. Therefore the hyphenation of separation methods, in the beginning mainly gas and liquid chromatography, with MS has gained more and more interest [13,14]. Especially the introduction of electrospray ionization (ESI) MS, which has greatly facilitated the interfacing of liquid phase separation methods to MS, has boosted this development. Coming back to capillary separation methods, there are several causes why these techniques are outstandingly suitable for the combination with MS detection, including their low flow rates which are well compatible with MS and the fact that detection sensitivity does not rely on a sufficiently wide capillary diameter as is the case with UV-detection.

This paper aims to discuss the various possibilities to hyphenate CEC with MS-detection and to give an overview regarding the increasing number of applications of this combination published within recent years.

2. Capillary electrochromatography

2.1. Separation principles and column technology

In general CEC can be seen as a hybrid technique combining the principles of liquid chromatography and capillary electrophoresis [4,15]. Focusing on the separation of neutral analytes, the electrophoretic portion of the separation mechanism is restricted to the use of the electroosmotic flow (EOF) instead of a mechanical pump driving the mobile phase (and with it the analytes) towards the detection end of the capillary. A well known advantage of this fact is the plug like flow profile of the EOF which leads to distinctly higher efficiencies than the parabolic flow profiles of pressure driven systems. Unfortunately flow rates can only be varied within a limited range when purely EOF driven systems are applied. To overcome this problem so-called pressure-assisted CEC (pCEC) has been developed [16]. This combination leads to a less distorted flow profile than HPLC (depending on the contribution of pressure and the EOF to the overall flow rate) and the possibility to manipulate flow rates within a wider range and thereby speed up analysis times. If charged solutes are present a truly combined separation mechanism is observed in CEC and the chromatographic separation is superimposed by the electrophoretic mobilities of the ionic molecules. Taking advantage of these principles, a variety of different types of analytes ranging from inorganic ions [17] to large biomolecules [18,19] have been separated by CEC so far.

Focusing on the column technology employed two basically different types of columns can be distinguished. These are columns which are entirely filled with a stationary phase and open tubular (OT) columns, where the stationary phase is only applied as a thin film on the capillary surface [20]. Although the majority of CEC applications are performed using the first type of columns, a number of applications involving OT-CEC columns can be found in the literature [21]. The recent advances in the fabrication and application of OT-CEC columns produced by a sol–gel process have been reviewed by Malik [22]. CEC columns which are entirely filled with the stationary phase can again be subdivided into two groups: packed bed columns and columns with a monolithic stationary phase. In particular, the advancement of monolithic stationary phases has been greatly boosted over the last years. This can clearly be seen from a series of review papers and book chapters published on this topic [4,23–25]. Another trend that can also be observed in CEC is the development of integrated micro-separation systems, e.g. on chips. Reports dealing with the fabrication and application of chips based on the principles of CEC can also be found in the recent literature [26–28].

2.2. Instrumentation and detection methods

Focusing on the instruments available commercially, only very few dedicated instrumentation for CEC can be found [29,30]. For this reason, CE instruments with only small modifications meeting the minimum requirements for their use in CEC are mostly widely employed. A major disadvantage of such instrumentation is the limited possibility to apply a pressure driven flow via an external device. For this reason, a wide range of home made CEC instruments has been described in the literature [16]. A principal advantage of these dedicated instruments (over the conventional CE

instruments) is the possibility to perform pCEC by application of an external pressure or an external flow via an HPLC pump. This allows to manipulate flow rates within a wide range and also gradient elution is made possible via this approach [31,32]. Regarding detection, CEC has been combined with a series of different detection methods [16]. Besides the most commonly employed UV-detector these include fluorescence [33] and laser induced fluorescence detection [34–36], NMR detection [37,38], conductivity detection [39] and finally MS detection [40]. In general UV-detection is not perfectly suited for capillary separation techniques as the very short optical path lengths involved lead to an unfavorable reduction in signal intensity, according to the law of Lambert and Beer. For this reason several attempts have been made to enhance the performance of this combination by the use of detection cells with extended light paths. CEC with UV-detection has been performed employing capillaries with so-called bubble cells [41] or the even more efficient Z-shaped cell [42]. Another approach is to enhance separation efficiency and to reduce band broadening caused by frits and the void space after the column packing using the so-called in-column detection, i.e. the measurement of UV absorbance across a part of the packed capillary [43].

In contrast to these spectrophotometric detection methods where according to their working principle considerably low mass detection limits are accompanied by relatively poor concentration detection limits MS detection does not suffer from this drawback. Apart from that, the combination of MS detection with capillary electroseparation methods provides a series of additional benefits. Spectrophotometric detection always depends on the corresponding characteristics of the solutes in particular their absorption coefficient or the ability to show fluorescence after excitation at a selected wavelength. Compatible mobile phases have to be UV-transparent at these excitation and/or detection wavelengths. Focusing on MS detection the first step towards the successful detection of an analyte is its proper ionization, which can be accomplished for almost every type of analyte using the appropriate ionization method. Similar as is the case with UV-detection, some restrictions regarding the use of mobile phases apply. Mobile phases compatible with MS detection should be volatile and should not interfere with the ionization of the analytes, i.e. not form any neutral complexes like ion-pairs. Unfortunately most CEC methods developed so far have been optimized with respect to the needs of UV-detection and are based on non-volatile electrolyte systems. Nevertheless, employing a MS interface with an additional sheath flow only moderately volatile mobile phases can also be used without detrimental effects on the MS performance due to the substantial dilution of the column effluent [44].

One of the most important advantages of MS detection over UV-detection is the possibility to achieve extended spectroscopic information on the nature of the analytes. These can be rather simple data like the mass of the solutes but also more comprehensive information on their structure if multiple stage MS is employed. Due to this additional knowledge certainty in peak assignment can be increased and the analyte peaks can be extracted from interfering background noise caused by unfavorable matrix components. If samples with restricted information about their composition have to be analyzed, MS^2 or even better MS^n experiments can provide useful information on the nature/structure of the included substances. In addition to these data acquired via the fragmentation of the analyte molecules in the MS, instrumentation with high mass accuracy like time of flight (TOF) or Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometers allows the exact determination of molecular masses and thereby can help to obtain information on the elemental composition of the investigated solutes.

A major difference between MS detection and UV-detection is the instrumental setup. Whereas UV-detection is generally performed in-column or on-column, MS detection is an end-column detection method. Therefore, because of the missing outlet buffer vial (in the case of MS detection) an alternative way to close the electronic circuit for the separation voltage is required. Additionally, decoupling of the separation voltage and the electronic circuit of the MS ion-source is beneficial. For this purpose a series of interfaces allowing the most efficient hyphenation of capillary electroseparation methods and MS detection have been designed, which will be discussed in detail in the following section.

2.2.1. Interfaces and types of MS instruments used for CEC–MS coupling

Since liquid phase separation methods have been coupled to MS, a series of different interfaces have been developed and tested for this purpose. Many of these interfaces, which were primarily designed for the hyphenation of liquid chromatography and MS, were consequently used for the coupling of CZE with MS and finally also for CEC–MS. Comprehensive overviews of the interfaces available for coupling liquid phase separation techniques to MS have been written by Gelpi [45] and Niessen [46]. According to this development it can be stated that the advancement of CEC–MS greatly benefited from the improvements in CZE–MS [47,48]. Combining capillary electroseparation methods with MS one is confronted with two major tasks: firstly, to transfer the analytes preferably completely from the capillary column into the MS orifice and secondly, an additional requirement specifically for electroseparation methods, to provide a proper grounding for the electrical circuit of the capillary column. Several different types of interfaces have been designed to meet these requirements [40].

The first interface ever used for the hyphenation of CEC with MS was the continuous flow fast atom bombardment (CF-FAB) interface [49]. Although this type of instrumentation allowed the detection of a wide range of polar and charged molecules it was affected with a series of drawbacks

in particular the relatively high chemical background (if low molecular mass solutes are monitored) as well as difficulties maintaining a stable electrical current [39]. Today this type of interface has been displaced by the more convenient atmospherical pressure ionization (API) interfaces. The most important exponents are the electrospray ionization (ESI) interface and the atmospheric pressure chemical ionization (APCI) interface [39,48]. In most setups the capillary column itself or a transfer capillary attached to the column are introduced into the electrospray needle of the ESI interface. Unfortunately most commercially available devices are equipped with relatively long sprayer needles requiring long columns and/or transfer capillaries and by this leading to unfavorably long analysis times. Therefore the demand to shorten column lengths is one of the driving forces for the development of lab made interfaces [50,51].

Focusing on ESI interfaces, the generation of gas phase ions from electrolyte ions is accomplished by creation of a strong electric field between the tip of the sprayer needle and the orifice of the MS instrument. In the consequently formed Taylor cone, the constant evaporation of the solvent from the droplets emerging from the sprayer tip accompanied by repeated coulombic fission and formation of smaller and smaller droplets finally leads to the release of the analyte ions [52]. A major advantage of ESI is the generation of multicharged ions, allowing the detection of high molecular mass substances using analyzers with a restricted *m/z* range. The nebulization process can be supported by a sheath gas supplied via an additional channel inside the sprayer needle. Focusing on the electric field needed for the electrospray ionization this can be achieved by two different setups. This is especially important as the type of setup used also determines the way how the two complete electrical paths for both the CEC and the ESI system are accomplished. The ideal arrangement is to maintain the column outlet at ground potential and to apply high voltage to the counter electrode of the MS system. For the realization of this approach a special design of the capillary depicted in Fig. 1 is required [52]. The crucial point of this setup is the isolation of the electrode in the vacuum from the ESI counter electrode in the atmospheric pressure part of the instrument. This can be accomplished by a glass capillary tube with a conductive coating on both ends. In this case the electric current flowing through the CEC column can be directly diverted via the grounded ESI tip. Unfortunately most MS instruments show a different design with the ESI voltage applied to the sprayer needle. In this case the current arising from the CEC system has to be transported through the gas phase to the grounded counter electrode of the ESI system. Depending on the current flowing through the column the resulting potential may be higher than the potential required for an optimum ESI process. As a consequence of this fact unwanted effects like poor sensitivity due to a reduced ionization of the analytes or arcing between the sprayer needle and the counter electrode may occur. To overcome this problem the column outlet and with it the sprayer needle can be grounded using a resistor of 40–100 MΩ [40].

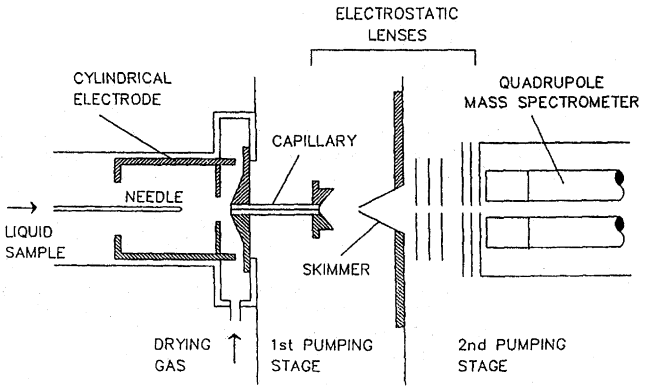

Fig. 1. Schematic of a Fenn–Whitehouse ESI source design. From [52] with permission.

A further classification of ESI interfaces suitable for the connection of CEC with MS can be obtained by the differentiation between (coaxial) sheath flow interfaces, liquid junction interfaces and sheathless interfaces as can be seen in Fig. 2. Smith et al. [53] introduced the coaxial sheath flow interface in 1988 which still is the most commonly employed species. The need for the addition of a sheath liquid arises from the combination of a method providing flow rates in the $nL\,min^{-1}$ range (CEC) with interfaces designed for flow rates in the $\mu L\,min^{-1}$ range. Mixing the column effluent with the sheath liquid can be accomplished either by the use of specially designed sprayer needle, which often also allows the addition of a sheath gas, equipped with two concentric tubes surrounding the capillary column or a simple T-piece before the electrospray tip. Benefits of this setup involving a sheath liquid are the formation of an electrical contact between the column outlet and the electrode, the increased stability of the electrospray and the possibility to employ mobile phases which only show a reduced compatibility with the ESI process. The latter fact arises from the strong dilution of the effluent by the sheath liquid. Using liquid junction interfaces, introduced by Henion et al. [54], the small gap between the separation capillary and the ESI emitter can be used to apply the ESI voltage but also to introduce additional liquids improving the spraying process. A further benefit of this setup is, that the separation capillary is partially decoupled from the ESI. The third type of interface frequently used for the hyphenation of CEC to MS is the sheahtless interface developed by Olivares et al. [55]. Nowadays sheathless interfaces are commonly employed in the form of the low flow electrospray or nano spray device developed by Wilm and Mann [56], which is especially suitable for the low flow rates in the range of $100\,nL\,min^{-1}$ present in CEC. Pre-requisite for such a setup are very sharp capillary tips with an internal diameter of a few micrometer and an electrically conducting coating on the surface or an electrical contact via an in-column electrode [57]. The first can be achieved either by the use of CEC columns with column ends prepared appropriately or the coupling of conventional columns to nano

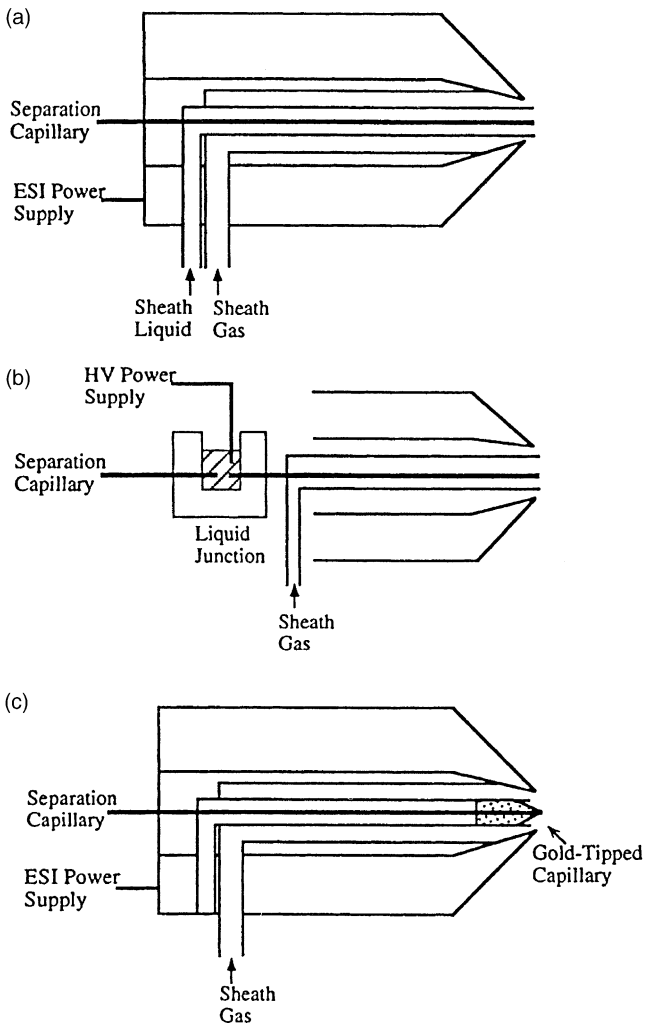

Fig. 2. Schematic of CE–MS interfaces to an ESI source: coaxial sheath flow interface (a); liquid junction interface (b); and sheathless interface (c). From [52] with permission.

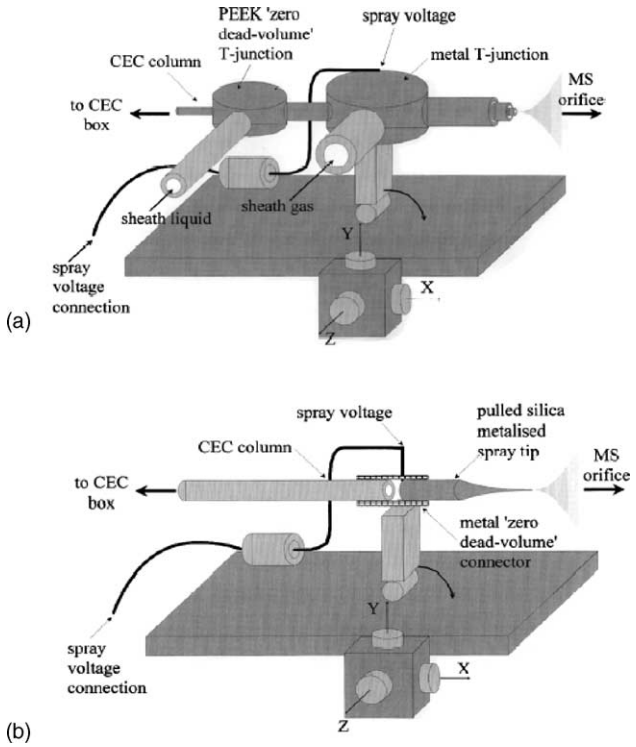

Fig. 3. Schematic of the CEC/microspray interface (a) and the CEC/nanospray interface (b). From [59] with permission.

spray tips. CEC columns with an integrated, conductive nano spray tip have been commercialized recently [58]. A principal drawback of the first approach is that the limited lifetime of the conductive coating requires the replacement of the whole column. A micro spray ESI interface with an additional sheath liquid at a flow range of 1 μL min^{-1} (Fig. 3a) and a sheathless nanospray interface (Fig. 3b) have been evaluated with respect to their suitability for the CEC–MS analysis of a steroid mixture by Warriner et al. [59]. Comparing the sensitivity obtained, the nano spray device was found to be 5–10 times more sensitive due to several reasons. Firstly a larger prortion of the ions produced in the ESI process actually find their way into the MS, because the nano spray tip is commonly positioned more closely to the MS orifice than other interfaces; secondly flow rates influence the size of the droplets formed, with smaller droplets (due to the reduced flow rate) leading to larger surface areas and with it improved ionization of the analytes; thirdly no dilution of the sample by the sheath flow occurs [48]. The layout of the interface used can also influence the peak shapes finally obtained. Even a high degree of chromatographic performance obtained from an almost perfect CEC separation can be turned into an unacceptable result by a suboptimal setup regarding the connection between the column outlet and the MS source. Recently this topic has been addressed by Boughtflower et al. [60]. They investigated the amount of peak dispersion originating from a series of different column-tube arrangements and offered valuable suggestions for the most practical way of connecting CEC–UV and MS detection without any major loss in separation performance.

Apart from these most commonly employed ESI interfaces, APCI interfaces would offer substantial advantages if medium to low polarity analytes have to be ionized. Nevertheless, almost no reports on the hyphenation of CEC with MS using an APCI interface exist [61]. A possible reason for this is that most APCI interfaces are designed for relatively high flow rates. An APCI interface capable of handling flow rates in the low μL min^{-1} range which might be suitable for combination with CEC, has been presented by Ozaki et al. [62]. Fig. 4 shows a further development of this interface [63]. A highly specific device for interfacing separation methods to MS is the inductively coupled plasma (ICP) interface. Its main field of work is the analysis of inorganic analytes and organic molecules containing metal ions including the speciation analysis of these metals [64,65]. In addition to these interfaces, actually employed in the hyphenation of electroseparation methods to MS in the present time, a series of further LC–MS or CZE–MS interfaces,

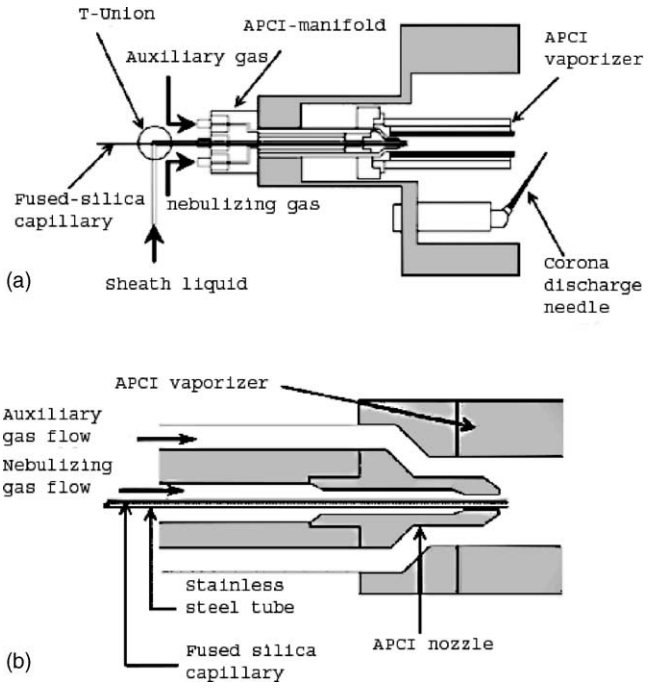

Fig. 4. Schematic diagram of a modified APCI interface for CZE-MS (a) and details of the coaxial sheath flow configuration (b). From [63] with permission.

based on different ionization principles, exist. Some of them might be of interest for CEC–MS in the future. A typical example is the atmospheric pressure photoionization (APPI) interface, a device allowing the ionization of substances only poorly amenable to ESI or APCI [66]. Chang et al. presented a so-called laser vaporization ionization (LVI) interface for CZE-MS coupling, depicted in Fig. 5. They employed a UV-laser for vaporization and ionization of the capillary effluent and a $CuCl_2$ solution acting as the carrier electrolyte and also as the matrix absorbing the energy from the laser [67]. From the point of view of separation science matrix assisted laser desorption/ionization (MALDI) MS is a typical off-line technique, offering several advantages if

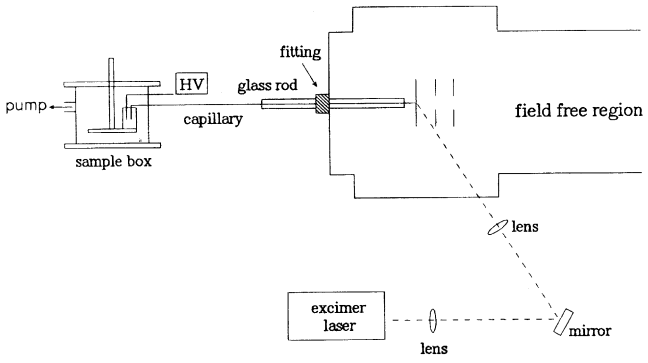

Fig. 5. Laser vaporization ionization interface. The CE effluent is directed to the MS instrument where it is vaporized and ionized by an UV laser. From [67] with permission.

the ionization of high mass analytes is required [45]. The combination of liquid phase separation methods with continuous flow MALDI-MS has been carried out by Nagra and Li [68]. Although, due to its flow rates in the low $\mu L\,min^{-1}$ range this setup would also be suitable for coupling with CEC, no reports of CZE- or CEC-MALDI-MS exist up to now.

Focusing on the MS part, it can be seen that almost all types of different MS instruments have been coupled to CEC [48]. According to the complexity of the analytical problem, the whole range of instrumentation from a simple single quadrupol MS, suitable as mass selective detector, to high performance spectrometers like FT-ICR instruments have been employed so far. Linear quadrupole mass analyzers have become extremely popular due to their low cost, small dimensions and the ease of operation by non specialist operators. Whereas single quadrupole instruments are mainly suitable as simple mass selective detectors and offer only limited options if comprehensive structural information on the analytes is required, modern triple quadrupol instruments provide the advantages of MS–MS experiments. Ion trap analyzer include the more commonly used three-dimensional ion trap and the revently developed linear ion trap. Due to their configuration they allow MS^n experiments and thereby provide additional information by multiple stage fragmentation of the analytes. These mass analyzers show a limited mass range (up to *m*/*z* 6000), medium to high resolution and a mass accuracy in the range of 100 ppm [40]. If increased resolution power and a highly accurate determination of molecular masses are required, reflectron time-of-flight (TOF) mass analyzers or the even more powerful FT-ICR instruments are needed. A further advantage of these instruments is their extended mass range allowing the analysis of large biomolecules. Taking into account the narrow peak widths achieved in CEC and the number of data points necessary to describe the peak sufficiently, the acquisition rate of the selected MS instrument also plays an important role. Especially TOF analyzers are characterized by their high data acquisition rate making them an optimum choice for high performance separation methods. To extend the possibilities of multiple stage MS to both TOF and FT-ICR analyzers, hybrid instruments with an additional linear quadrupole or an ion trap have been developed [69,70].

3. Applications of CEC–MS

Browsing through the literature, a slight change in the type of papers published on CEC–MS could be observed within the last few years. Whereas in the early times the main focus was set on the further development of the instrumental setup allowing the hyphenation of this relatively new separation technique with MS, more and more applications of this combination in a variety of fields (however with the main emphasis set on bioanalytical applications) can be

found in the recent literature. In the following section applications of CEC–MS, classified according to the type of CEC columns employed (either packed/monolithic columns or OT columns) are reviewed. A short summary including the essential information on the type of instrumentation employed as well as on the separation parameters used is given in Table 1. To keep the selection up-to-date, only the more recent literature, published since 1998, has been included.

3.1. Packed columns and monolithic columns

In the beginning of CEC, mostly packed columns were used. Unfortunately this type of separation media was affected with a series of problems including the fragility of the columns and obstacles related to the fabrication of frits as well as their tendency towards clogging during use. For this reason continuous bed or monolithic columns have gained more and more importance in CEC over the years. Their major advantages are as follows: they can be produced more easily than packed ones and monolithic columns do not require frits which have always been a major drawback of conventional CEC columns. The disadvantage of frits is not only because of to the well known mechanical problems involved (breaking, clogging, etc.) but also due to the frequent formation of gas bubbles caused by the different physico-chemical properties of the packing material and the retaining frits. Whereas the latter problem could be reduced in CEC–UV by pressurizing the inlet and the outlet buffer vial, this approach is not possible in CEC–MS. A potential way to overcome some of the limitations of packed columns in CEC–MS has been described by Lord et al. [71]. The use of tapers and restrictors in place of frits prior to the MS detector showed potential in suppressing bubble formation which is often encountered when conventional outlet frits are used. Despite these attempts to minimize some of the problems encountered in CEC–MS with conventionally packed capillary columns, an increasing number of applications employing monolithic columns can be found in the literature. Unfortunately the latter type of columns is not yet available commercially, so scientists still rely on lab-made devices.

3.1.1. CEC–MS analysis of proteins, peptides and amino acids

A number of papers dealing with the CEC–MS analysis of proteins, peptides (mainly from digests) and also amino acids have been published within the last 5 years. These include the only report (up to now) presenting a micro fluidic device for CEC–MS [72]. The system with a channel length of 5–6 cm provided separation efficiencies in the range of 3000–4000 plates, which did not allow the complete separation of all peptides originating from a bovine haemoglobin digest within a 10–12 min time window. Commonly a co-migration of two to three peptides occurred but the CEC–MS system resulted in a much simplified spectrum for the peptides, compared to those from simple infusion experiments. This is due to the ability of this miniaturized separation system to provide at least partial resolution for most of the analytes. An additional benefit, leading to improved detection limits is the reduction of problems associated with signal suppression originating from co-elution of analytes and matrix components. pCEC is a very common technique which helps to reduce problems associated with bubble formation in CEC (e.g. instable separation current) and allows to generate a more constant flow from the outlet of the CEC into the source of the MS. Finally the addition of a pressure driven flow gives the opportunity to manipulate separation velocity in a wider range than in purely EOF driven systems. Ivanov et al. presented an investigation on the suitability of pCEC with UV and ESI-MS detection for the highly efficient separation of peptide mixtures [73]. Plate numbers of up to 300 000 plates m^{-1} were obtained employing custom made monolithic columns prepared from in situ polymerized methacrylic monomers in the presence of different porogens. Gaspari and co-workers and Guèek and co-workers published two papers dealing with the performance of two different interfaces for CEC–MS (with and without additional sheath flow) with respect to the separation and detection of peptides down to the attomole level [74,75]. The lab-made nanospray interface for CEC–MS coupling provided a stable spray without any sheath liquid being employed [74]. Nevertheless the system suffered from an undesirable connection between detection sensitivity and separation voltage. The authors assumed that an increase in current due to a higher separation voltage led to bubble formation and with it a decrease in detection sensitivity. A following work by this group investigated the same set of analytes employing CEC–MS with an ESI interface and an additional sheath liquid [75]. In the case of this experimental setup no interference of the detection sensitivity by the separation voltage applied was observed. For this reason the authors recommended the use of sheath flow interfaces for CEC–MS as they allow to choose the separation voltage within a wider range and thereby lead to decreased analysis times. Walhagen et al. described the design and construction of an injection valve for pCEC–MS with a rotating injection port including compartments for the eluent as well as the sample and its application to the analysis of a set of peptides [76]. Electrically assisted LC for the separation of peptide digests in the isocratic as well as the gradient mode is presented in a work by Apffel et al. [77]. Chromatograms obtained in the pure LC mode have been compared with experiments where different voltages (either positive or negative) were employed and an additional electrophoretic effect was observed as can be seen from Fig. 6. Detection was performed with an UV-detector and a single quadrupol-MS. Huang et al. published two papers on the separation of peptide mixtures by pCEC coupled to an ion trap storage reflectron TOF-MS [78,79]. Several peptides were separated using 150 μm i.d. capillaries packed with either C_{18} silica [78] or a mixed-mode C_{18}/strong anion-exchange (SAX) stationary phase [79]. The latter material provided improved performance as it allowed selectivity tuning with

Table 1
Applications of CEC–MS

Sample	Separation conditions	Interface mass analyzer	Sheath liquid	Ref.
Packed column and continuos bed CEC				
Proteins, peptides and amino acids				
Peptide digests	Polymer monoliths, microfluidic device 15 mM NH_4Ac (pH 4.6)–MeOH (7:3)	ESI-TOF-MS		[72]
Peptide digests	Polymer monoliths, 19–26 cm × 50 μm i.d. 20 mM NH_4Ac (pH 4.4)–ACN (8:2) 2.4 mM NH_4Ac (pH 9.4)–ACN (1:1) 25 mM Tris–HCl (pH 4.0)–ACN (4:6) 0.1% HAc (water)/0.1% HAc (ACN), gradient	ESI-ion-trap-MS ESI-TOF-MS		[73]
Peptides	Hypersil C_{18}, 25 cm × 100 μm i.d. 3.2–3.4 mM NH_4Ac (pH 6.7)–ACN (50:50)	ESI (nanospray)-ion trap-MS		[74]
Peptides	Hypersil C_{18}, 33.5 (25) cm × 100 μm i.d. 6.4 mM NH_4Ac (pH 6.7)–ACN (50:50)	ESI-ion trap-MS	HAc–MeOH–water (0.1:80:20) at 1–1.5 μL min^{-1}	[75]
Peptides	Hypersil C_{18}, 33.5 (25) cm × 100 μm i.d. 6 mM NH_4Ac–ACN (50:50)	ESI-ion trap-MS	HAc–MeOH–water (0.1:80:20) at 1–1.5 μL min^{-1}	[76]
Peptide digests	Vydac 218 TPB5 RP_{18}, 25 cm × 100 μm i.d. 0.1% TFA in water, 0.09% TFA in ACN gradient	ESI-single quad MS	50% HAc at 3–5 μL min^{-1}	[77]
Tryptic digests	Vydac C_{18} 8.5–12 cm × 150 μm i.d. 0.04 % TFA in water/0.04 % TFA in ACN, gradient	ESI-ion-trap-storage/TOF-MS		[78]
Peptide mixtures	Vydac C_{18}, C_{18}/dialkylamine, 8.5–12 cm × 150 μm i.d. 0.014% TFA in ACN–water (25:75) 4 mM HAc/NH_4Ac ACN–water (25:75)	ESI-ion trap-storage/TOF-MS		[79]
PTH-amino acids	Zorbax C_{18} 8.5–12 cm × 75 μm i.d. 2 mM NH_4Ac in water/2 mM NH_4Ac ACN–water (9:1), gradient	ESI-TOF-MS	0.2 mM NH_4Ac in MeOH–water (9:1) at 3 μL min^{-1}	[50]
Saccharides				
Glycan mixtures from glycoproteins	–CN polymer monoliths, 26 cm × 100 μm i.d. 2.4 mM NH_4Ac (pH 3), 0.2 mM NaAc in ACN–water: [(50–71):(50–29)]	ESI (nanospray)-FT-ICR-MS	1% FA, 1 mM NaAc in ACN–water (1:1) at 0.5 μL min^{-1}	[80]
Isomeric oligosaccharides	–CN and –NH_2 polymer monoliths, 22–28 cm × 100 μm i.d. ACN–water–240 mM NH_4 formate: [(80–55):(44–19):1]	ESI-ion trap-MS	1% FA, 1 mM NaAc in ACN–water (1:1) at 0.5 μL min^{-1}	[81]
Neutral saccharide mixtures	–CN and –NH_2 polymer monoliths, 22–28 cm × 100 μm i.d. ACN–water–240 mM NH_4 formate: [(95–60):(39–4):1]	ESI (nanospray)-ion trap-MS	1% FA, 1 mM NaAc in ACN–water (1:1) at 0.5 μL min^{-1}	[82]

Chiral compounds				
Warfarin enantiomers	(3*R*,4*S*)-Whelk-O1 CSP 60 (59) cm × 75 μm i.d. 2.5–10 mM NH_4Ac (pH 3–5) in ACN–water: [(60–80):(40–20)]	ESI-single quad-MS	5 mM NH_4Ac (pH 6.8) in MeOH–water (1:1) at 5 μL min^{-1}	[85]
Drug enantiomers	Chira-Dex-silica, 30 cm × 100 μm i.d. 0.5 mM NH_4Ac–MeOH [(70–40):(60–30)]	CIS-triple quad-MS and ESI-triple quad-MS	ESI: water at 3 μL min^{-1} CIS: $AgNO_3$ or LiI (100 μg ml^{-1}), $CoCl_2$ (160 μg ml^{-1}), $CuCl_2$ (80 μg ml^{-1}) at 3 μL min^{-1}	[86]
Miscellaneous applications				
Withanolides from plant extracts	Hypersil C_{18}, 33.5 (25) cm × 100 μm i.d. 20 mM FA–NH_4OH–ACN (1:1)	ESI-single quad-MS	0.5% FA 2-propanol–water (1:1) at 3 μL min^{-1}	[87]
Extracts from ergot fungus	Grom-Sil ODS-0 AB or Grom-Sil 120 SEC, 25 cm × 250 or 100 μm i.d. ODS: 99% water to 99% ACN in 240 min SEC: (79.95:20:0.05) water–ACN–FA	CIS-triple quad-MS		[88]
Fatty acid methyl esters, vitamins and estrogens	Grom-Sil ODS-0 AB 25 cm × 100 μm i.d. 40 mM NH_4Ac (pH 9) in ACN–water (95:5) 20 mM NH_4Ac (pH 9) in ACN–water (90:10) 4 mM NH_4Ac (pH 9) in ACN–water (50:50)	CIS-triple quad-MS	100 μg ml^{-1} $AgNO_3$ in water at 3 μL min^{-1}	[89]
Flunitrazepam and metabolites	Hypersil C_{18}, 33.5 (25) cm × 100 μm i.d. 25 mM FA/NH_4OH (pH 8)–ACN (40:60)	ESI-single quad-MS	0.5% FA in MeOH–water (1:1) at 5 μL min^{-1}	[90]
Non-steroidal anti-inflammatory drugs	LiChrospher RP_{18}, 53 cm × 100 μm i.d. 25–150 mM FA (pH 2.5 with NH_3)–ACN (50–80%)	ESI-ion trap-MS	1% NH_3 in MeOH–water (70:30) at 5 μL min^{-1}	[91]
Etodolac and metabolites in urine	LiChrospher RP_{18}, 33 (23) cm × 100 μm i.d. 10 mM NH_4 formate (pH 3)–ACN (1:1)	ESI-ion trap-MS	10 mM NH_4 formate (pH 3)–ACN (1:1) at 3 μL min^{-1}	[92]
Etodolac and metabolites in biological samples	LiChrospher RP_{18}, 33 (23) cm × 100 μm i.d. 10 mM NH_4 formate (pH 3)–ACN (1:1)	ESI-ion trap-MS	10 mM NH_4 formate (pH 3)–ACN (1:1) at 3 μL min^{-1}	[93]
Plasma samples	Hypersil Duett C_{18}/SCX 40 (25) cm × 100 μm i.d. 25 mM NH_4Ac (pH 4)–ACN (80%)	ESI-triple quad-MS	0.1 % HAc in MeOH–water (70:30) at 2 μL min^{-1}	[94]
Steroids	–C_{12} polymer monoliths, 35 cm × 100 μm i.d. ACN–water–240 mM NH_4 formate (pH 3) (60:45:5)	ESI-ion trap-MS	1% FA, 1 mM NaAc in ACN–water (1:1) at 0.5 μL min^{-1}	[95]
Biological samples	Nucleosil RP_{18}, 37 (16) cm × 75 μm i.d. LE: 20 mM NH_4Ac (pH 5) in MeOH–water (75:25) TE: 20 mM β-alanine (pH 5) in MeOH–water (75:25) (sample precocentration via ITP-CEC coupling)	ESI-single quad-MS	1% HAc in MeOH–water (8:2) at 1 μL min^{-1}	[96]
Drug standards	Spherisorb C_{18}, C_6/SCX and SCX, 45 cm × 100 μm i.d. 25 mM NH_4Ac (pH 4)–ACN (2:8)	ESI-triple quad-MS	0.1% HAc in MeOH–water (7:3) at 2 μL min^{-1}	[97]
Corticosteroids	Spherisorb C_{18}or C_6/SCX 37 cm × 50 μm i.d. 20 mM NH_4Ac (pH 4)–ACN (30:70)	ESI-triple quad-MS	0.1% HAc in MeOH–water (7:3) at 4 μL min^{-1} (only ESI)	[98]
Mixture of pharmaceuticals	Methacrylate-based nanoparticles (diameter 160 nm) in 75 cm × 50 μm i.d. capillary 50 mM NH_4CO_3 (pH 8.2)–ACN	ESI-ion trap-MS	MeOH–water (1:1) with 0.1% FA	[99]
Bile acids	–C_{12} and –NH_2 polymer monoliths, 22–28 cm × 100 μm i.d. ACN–water–240 mM NH_4formate (pH 3) [(60–55):(40–35):5]	ESI-ion trap-MS	1% FA in ACN–water (1:1) at 0.5 μL min^{-1} (pos mode) 10 mM NH_4Ac (pH 7)–ACN (1:1) at 0.5 μL min^{-1} (neg mode)	[100]

Table 1 (*Continued*)

Sample	Separation conditions	Interface mass analyzer	Sheath liquid	Ref.
Pyrimidine derivatives	Hypersil C_{18}, 48 (40) cm × 100 μm i.d. 8 mM NH_4Ac (pH 6)–ACN (60:40), 20 mM *p*-benzoquinone	ESI-single quad-MS	1% HAc isopropanol–water (8:2) at 4 μL min^{-1}	[101]
PAH-DNA adducts	Hypersil C_{18}, 20 cm × 75 μm i.d. 5 mM NH_4Ac–MeOH–ACN (60:30:10) 5 mM NH_4Ac–MeOH–ACN (49:35:16) 5 mM NH_4Ac–MeOH–ACN (34:50:16) step gradient	ESI-triple quad-MS	1% HAc in MeOH–water (75:25) at 0.8 μL min^{-1}	[102]
Nucleoside adducts	Hypersil C_{18}, 40 (25) cm × 75 μm i.d. 5 mM NH_4Ac (pH 4)–ACN–MeOH (60:10:30)	ESI-triple quad-MS	1% HAc in MeOH–water (7:3) at 0.5–0.8 μL min^{-1}	[103]
Surrogate tags	Spherisorb C_{18}/SCX 45 cm × 50 μm i.d. 25 mM NH_4Ac (pH 4)–ACN (2:8)	ESI-triple quad-MS	0.1% FA in MeOH–water (8:2) at 3 μL min^{-1}	[104]
Open tubular CEC				
Hexobarbital enantiomers in urine	Chirasil-Dex coating 45 (25) cm × 50 μm i.d. 10 mM NH_4Ac (pH 7.2)	ESI-triple quad-MS	10 mM NH_4Ac (pH 7)–isopropanol (9:1)	[104]
Metal speciation	Macrocyclic polyamine coating 160 cm × 100 μm i.d. 20 mM phosphate (pH 6.2)	ICP-singe quad-MS		[105]

ESI: electrospray ionization; FT-ICR-MS: Fourier transform ion cyclotron resonance mass spectrometer; single quad-MS: single quadrupole mass spectrometry; triple quad-MS: triple quadrupole mass spectrometry; TOF-MS: time of flight mass spectrometry; pos mode: positive ion mode; neg mode: negative ion mode; Ac: acetate; HAc: acetic acid; MeOH: methanol; Tris: trishydroxymethylaminomethane; ACN: acetonitrile; TFA: trifluoroacetic acid; FA: formic acid; PTH: phenylthiohydantoin; LE: leading electrolyte; TE: terminating electrolyte; ITP: isotochophoresis.

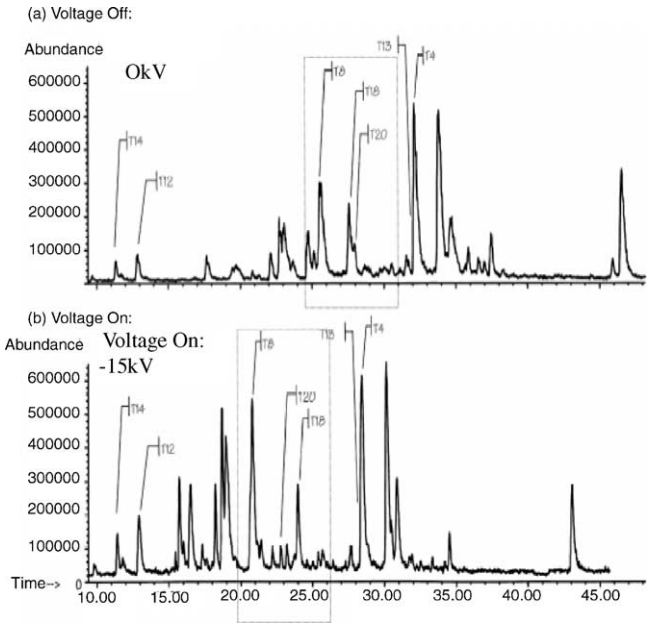

Fig. 6. Tryptic digest of recombinant human growth hermone (rhGH) cLC–MS (a) and by electrically assisted cLC–MS (b). Column: 25 cm × 100 mm i.d. 5 mm Vydac C_{18}. Mobile phase (A) 0.1% trifluroacetic (TFA) in water; (B) 0.09% TFA in acetonitrile. Gradient: initial 0% for 5 min; 0–60% (B) in 60 min. From [77] with permission.

electrolytes in the pH range between pH 2–5, where the ionic sites of the SAX groups (in contrast to conventional C_{18} material) still provide a substantial EOF. Choudhary et al. presented a comprehensive work dealing with the analysis of biomolecules, in particular phenylthiohydantoin amino acids, by CEC–TOF-MS [50]. They investigated a wide range of parameters including sheath liquid composition, column length, isocratic versus gradient elution with different gradient slopes and applied voltage. Finally 12 phenylthiohydantoin amino acids could be separated in less than 8 min employing gradient CEC with MS detection.

3.1.2. CEC–MS analysis of saccharides

Several papers on the CEC–MS analysis of saccharide mixtures were published by Novotny and co-workers [80–82]. Two high performance methods, namely CEC and FT-ICR-MS have been employed to deal with the complexity of the glycan mixtures released from glycoproteins like bovine mucin and bile-salt-stimulated lipase (BSSL) [80]. The very high overall resolving power obtained by combining the outstanding spatial resolution of CEC and the excellent mass resolution of FT-ICR-MS allowed the identification and structural determination of a large number of these glycans. Using CEC with polar monolithic columns and MS–MS detection employing an ion-trap MS the same group succeeded in the structural characterization of neutral oligosaccharide mixtures released from Ribonuclease B and BSSL [81]. The tandem spectra allowed to discriminate between isomeric oligosaccharides with a different monomeric composition and branching. In an earlier published paper, a similar approach was employed to demonstrate the feasibility of CEC–MS in carbohydrate analysis on the hand of malto-oligosaccharides [82]. Generally CEC separations combined with ESI-MS detection showed substantial improvements over analytical methods solely depending on the resolution power of MS, like the widely used MALDI-MS techniques. This is particularly important when it comes to the analysis of complex mixtures as encountered in the case of glycans released from glycoproteins.

3.1.3. CEC–MS analysis of chiral compounds

Packed column or monolithic column CEC has also been applied to the separation of chiral compounds as can be seen from several review papers dealing with this issue [83,84]. Zheng and Shamsi showed the suitability of CEC–MS using a column packed with a commercially available stationary phase for the determination of warfarin and coumachlor enantiomers in human plasma [85]. Benefits of the developed method are its minimal sample and mobile phase consumption and the ability to differentiate between a minor enantiomer (as little as 1%) in excess of the major enantiomer (up to 99%). An interesting way to allow the acquisition of MS signals also for analytes that usually cannot be easily detected by ESI-MS has been developed by Brocke et al. The so-called coordination ion spray (CIS) mass spectrometry involves the use of metal ions, usually added to the sheath liquid as salts, forming charged complexes with these compounds. This approach has also been employed in the CEC–MS analysis of a series of chiral compounds [86]. A CEC column packed with Chira-Dex-silica was used to separate enantiomers of barbiturates and chlorinated alkyl phenoxypropanoates. MS detection of these analytes was significantly improved in the CIS mode ($CoCl_2$ added to the sheath flow) compared to the normal ESI mode, as can be seen from Fig. 7.

3.1.4. CEC–MS analysis of other substances

CEC–MS has also been employed to the analysis of a wide range of solutes originating from different fields of application including investigations on extracts from plants [87], extracts from fungi [88], fatty acids, vitamins and estrogens [89], various types of drugs and related substances [59,90–99], bile acids [100], pyrimidines [101], adducts of polycyclic aromatic hydrocarbons with DNA [102] and styrene oxides with nucleosides [103], and dansylated secondary amine tags [51].

Cherkaoui et al. developed two methods based on either pseudo-stationary phases (MEEKC) or true stationary phases (CEC) for the analysis of withanolides in plant extracts [87]. Although the MEEKC method was found to be suitable for this analytical problem, this separation technique does not allow direct coupling with MS mainly due to the presence of large amounts of non-volatile ingredients like SDS in the mobile phase. For this reason a CEC–MS method was developed simultaneously, to obtain additional spectral information and thereby improve the certainty of

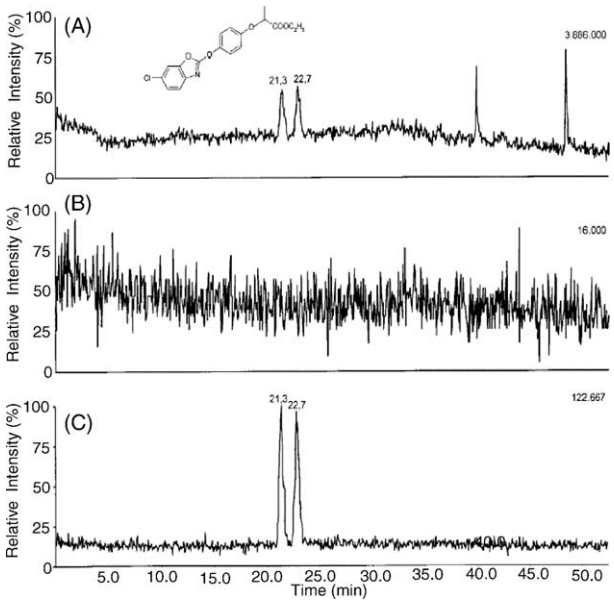

Fig. 7. Comparison of the detection of fenoxaprop ethyl enantiomers complexed with $AgNO_3$: total ion chromatogram (A); extracted ion chromatogram of $[M + H]^+$ (B); extracted ion chromatogram of $[M+^{107}Ag]^+$ (C); 30 cm × 100 μm i.d. column packed with Chira-Dex-silica. Conditions: 20.5 kV; 10 bar; mobile phase: 0.5 mM ammonium acetate buffer in water–methanol (40:60) at pH 6.6. From [86] with permission.

peak assignment. Cahours et al. employed a similar approach for the determination of flunitrazepam (FNZ) and its major metabolites [90]. Coupling of pCEC with CIS-MS was employed to the analysis of extracts from ergot fungus [88] and a variety of biologically relevant substances [89]. For the separation of the fungi extracts a series of interesting instrumental setups were developed and tested. Besides common pCEC using columns with a reversed phase packing, size-exclusion chromatography (SEC) under pCEC conditions is described for the first time in this paper. The short SEC columns used had two major disadvantages: their peak capacity was rather low and relatively concentrated samples had to be used. Nevertheless the presented pCEC–SEC-MS system could be employed for on-line desalting, separation and measurement of samples in a single step [88]. In a second paper CEC–CIS-MS, pCEC–CIS-MS and HPLC–CIS-MS were compared with respect to their suitability for the analysis of fatty acid methyl esters, lipophilic vitamins and estrogenic compounds [89]. The wide range of solutes which may be ionized by CIS-MS makes this technology more generally applicable than other spray techniques. Desiderio and Fanali investigated the separation and detection of non-steroidal anti-inflammatory drugs using CEC–MS [91]. Method optimization was performed with respect to a series of parameters, including composition of the mobile phase (amount of organic solvent, buffer concentration, buffer pH, etc.) and separation temperature. Strickmann et al. published two papers on the determination of another non-steroidal anti-inflammatory drug (Etodolac) and its urinary metabolites with CEC–ESI-MS [92,93]. Mixed-mode CEC with a C_{18}/strong cation-exchange (SCX) column coupled to a triple quadrupol MS was employed by Spikmans et al. for the analysis of complex plasma samples [94]. The column provided high separation efficiency and no decrease in column performance together with only minor changes in retention times were observed even after the analysis of a few hundred plasma samples.

Que et al. used gradient elution CEC to determine steroid profiles of urine samples [95]. Focusing on detection, MS detection with an ion-trap MS and laser-induced fluorescence (LIF) detection were compared with detection limits in the attomole range (LIF) and femtomole range (MS), respectively. Due to the very low concentrations of the selected steroids in the real samples, the application of CEC–MS would require additional sample pre-treatment before analysis. A way to overcome this problem and to facilitate the analysis of highly diluted samples by CEC–MS was presented by Mazereeuw et al. [96]. On-line isotachophoretic sample focusing allowed the detection of several drug compounds in urine and plasma down to the low ng L^{-1} level. A linear calibration curve could be constructed over three orders of magnitude for the selected low molecular-mass compounds. The trend towards a high degree of automation in analytical systems has also reached CEC–MS. Two papers focusing on this issue have been published by Lane and co-workers [97,98]. An interesting study describing the use of columns filled with a suspension of nanoparticles for the CEC–MS analysis of a drug standard was presented by Viberg et al. [99]. Two different approaches, partial filling of the column (so the nanoparticles do not reach the MS interface) as well as continuous filling were tested. Employing an orthogonal MS interface no interference from the nanoparticles was observed, even when continuously filled columns were used, as depicted in Fig. 8. Bile acids and their conjugates were analyzed using CEC–MS by Que et al. [100]. Monolithic columns with different surface chemistry, allowing to modify the magnitude and even the direction of the EOF, coupled to ion-trap MS were found to be useful for the separation and identification of bile acids in biological mixtures. Ahrer et al. reported the separation and detection of a series of pyrimidine derivatives, used as building blocks for the manufacturing of important herbicides and fungicides, by CEC–MS [101]. The interface used in this work included a metal transfer capillary which also acted as the cathode of the CEC system. An unwanted side effect of this setup is the formation of hydrogen bubbles in this transfer capillary (originating from the reduction of protons) which led to an unacceptable baseline noise. The addition of an easily reducible substance (in this case *p*-benzoquinone) to the carrier electrolyte helped to overcome this problem. Adducts of DNA and polycyclic aromatic hydrocarbons [102] as well as nucleosides with styrene oxide [103] were analyzed by Vouros et al. using CEC–MS. In the latter paper the feasibility of CEC for the differentiation of a charged nucleoside adduct from its neutral nucleoside analogue was demonstrated. Coupling of CEC to microspray ESI-MS even

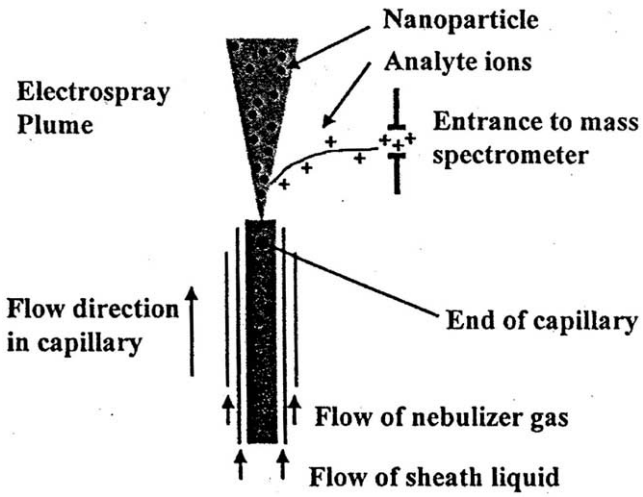

Fig. 8. Schematic illustration of the orthogonal ESI interface between the CEC and MS employed for CEC–MS with a nanoparticle pseudostationary phase. Positive ions are pulled out of the electrospray plume and accelerated in an electric field toward the inlet of the MS system. From [99] with permission.

allowed the detection of trace impurities which could not be observed by either HPLC–UV or CEC–UV. The application of CEC–MS in encoded combinatorial organic synthesis was reported by Lane and Pipe [51]. The high separation efficiency of CEC together with the inherent specifity of triple quadrupole MS allowed the unambiguous decoding of active single beads from encoded combinatorial synthesis.

3.2. Open tubular columns

Although the majority of CEC–MS applications still involve the use of packed or monolithic column, a few examples of OT-CEC with MS detection can be found in the recent literature. Schurig and Mayer reported the separation of hexobarbital enantiomers in spiked urine employing a short Chirasil-Dex coated column and a triple quadrupol MS in the selected reaction monitoring mode for detection [104]. An application of OT-CEC in the field of inorganic analysis was published by Chen et al. [105]. A bonded phase capillary column functionalized with macrocyclic polyamine groups was employed for the separation of arsenic, chromium and selenium species. Detection was performed with an inductively coupled plasma MS. The results obtained with this OT-CEC method were compared to those achieved with a bare fused silica capillary in the CZE mode. These two methods led to distinctly different migration/elution orders for the selected analytes.

4. Conclusions

This review shows that CEC–MS has developed from a relatively exotic technique, with most reports addressing mainly technical issues like the construction of suitable interfaces for the hyphenation of these two methods, into an analytical technique which is more and more applied to real world problems. Several major trends can be observed, browsing the literature published within the last few years. Firstly, the use of monolithic or continuous bed columns in CEC becomes more and more popular as this type of columns provide a series of benefits over the conventionally packed ones. Secondly, regarding the type of interface employed, nanospray interfaces which are better suited for the low flow rates observed in CEC and therefore can be operated without the addition of a sheath liquid have gained increased interest when it comes to the hyphenation of CEC with MS detection. Finally, the type of MS instrumentation used becomes more and more sophisticated. A growing number of CEC separations with reflectron TOF, FT-ICR-MS or hydride instruments involving these two technologies can be found in the literature.

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