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REVIEW

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Capillary electrophoresis mass spectrometry and its application to the analysis of biological mixtures

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Abstract Capillary electrophoresis (CE) mass spectrometry (MS), with its ability to separate compounds present in extremely small volume samples rapidly, with high separation efficiency, and with compound identification capability based on molecular weight, is an extremely valuable analytical technique for the analysis of complex biological mixtures. The highest sensitivities and separation efficiencies are usually achieved by using narrow capillaries (5–50 μm i.d.) and by using sheathless CE-to-MS interfaces. The difficulties in CE-to-MS interfacing and the limited loadability of these narrow columns, however, have prevented CE-MS from becoming a widely used analytical technique. To remedy these limitations, several CE-MS interfacing techniques have recently been introduced. While electrospray ionization is the most commonly used ionization technique for interfacing CE-to-MS, matrix assisted laser desorption ionization has also been used, using both on-line and off-line techniques. Moreover, the high concentration detection limit of CE has been addressed by development of several sample concentration and sample focusing methods. In addition, a wide variety of techniques such as capillary zone electrophoresis, capillary isoelectric focusing, and on-column transient isotachophoresis have now been interfaced to MS. These advances have resulted in a rapid increase in the use of CE-MS in the analysis of complex biological mixtures. CE-MS has now been successfully applied to the analysis of a wide variety of compounds including amino acids, protein digests, protein mixtures, single cells, oligonucleotides, and various small molecules relevant to the pharmaceutical industry.

Keywords Capillary electrophoresis · Electrospray ionization · Mass spectrometry · Protein analysis

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Introduction

High speed identification of biological molecules in complex mixtures of limited volume requires high separation efficiency and precise analyte identification with high sensitivity. Modern capillary electrophoresis (CE) has rapidly matured into a useful and accepted analytical technique offering a number of practical advantages, including high separation efficiency (up to 10^7 theoretical plates), high speed, and economy of sample size (a few nanoliters or less). In addition, modern mass spectrometry (MS) is capable of detecting a variety of compounds with a very high degree of selectivity (based on molecular weight information) without compromising sensitivity, dynamic range, or analysis speed. The combination of MS detection with a high-efficiency separation technique such as CE provides a powerful system for the analysis of complex biological mixtures.

High separation efficiency and high sensitivity at high analysis speeds requires that CE parameters be optimized for maximum resolution and sensitivity under conditions that are suitable for the ionization technique utilized. Moreover, the CE-to-MS interface must be robust, sensitive, easy to use, and able to maintain the optimum performance parameters of the CE. In addition, the mass spectrometer must provide accurate identification and quantification of analytes with a high degree of selectivity and sensitivity at speeds compatible with the narrow peaks generated under high performance CE.

Capillary electrophoresis

Capillary zone electrophoresis (CZE or simply CE) is the most general and basic state in CE [1, 2, 3]. With CE, separations occur inside a capillary filled with a moderately conductive liquid. The degree of separation of the analytes inside the capillary depends on their relative electrophoretic mobilities (μ_{ep}, $cm^2/V{\cdot}s$) under the applied electrical field,

$$\mu_{ep} = \nu_{ep}/E = q/6p\eta r \quad (1)$$

where ν_{ep} is electrophoretic (ionic) velocity, E is the field strength ($E=V/L$, V=separation voltage, and L=column length), q is the analyte's net charge, η=viscosity of the buffer, and r=ionic radius. An analyte's electrophoretic mobility is a function of its charge/size ratio. Size is a function of mass, shape, and degree of solvation. Small, highly charged species usually have large electrophoretic mobilities, while large, minimally charged species have lower mobilities. Metal ions are exceptions since smaller ions are usually more hydrated and, therefore, have lower ionic mobilities. In the absence of electroosmotic flow (EOF), $\nu_{ep}=L/t_m$, where t_m is the migration time. However, because the observed ν_{ep} usually includes the electroosmotic velocity (υ_{eo}), to calculate μ_{ep} the value of υ_{eo} must be known.

The most commonly used material for the separation capillary is fused silica. Dry fused silica capillaries have silanol groups [Si-O-H, with an isoelectric point (pI) of approximately 2] on their inner surface. However, once the capillary is filled with a buffer solution, the inner wall of the fused silica capillary becomes charged according to acid/base reactions between the solid and solvent. [In this article the word buffer solution refers to a weakly conductive solution used as a background electrolyte (BGE) that may or may not have buffering capacity]. These reactions determine the polarity of the charge imposed on the wall. For fused silica, an aqueous BGE with a pH>pI will impose a negative charge on the silanol groups.

The negatively charged silanol groups attract positively charged ions of the BGE, forming an electrical double layer. The positive ions (cations) include those attached to the wall (fixed cations) and those that are mobile and hydrated in solution. These hydrated cations in the solution include a compact layer of mobile ions with a net positive charge that forms a thin (~10 nm) charged sheath around a core of uncharged liquid (this layer is responsible for the EOF) and a diffuse layer in which the net positive charge falls exponentially to zero with respect to the distance from the surface. Application of a potential gradient across the length of the capillary creates shear within the sheath, generating EOF towards the negative electrode. Hydrogen bonding of water molecules or Van der Waals interactions between BGE constituents across the diameter of the capillary transmits the EOF, forming a plug-shaped flow profile. The Helmholtz-Smoluchowski equation gives the linear velocity of the EOF (ν_{eo}):

$$\nu_{eo} = \varepsilon\xi E/\eta \quad (2)$$

where ε is the BGE's dielectric constant, and ξ is the zeta potential of the liquid-solid interface. The zeta potential is given by $\xi=4\pi\delta e/\varepsilon$, where δ=double layer thickness (Debye ionic radius) and e=total excess charge in solution per unit area. The double layer thickness is equal to $\delta=[3\times10^7|Z|C^{1/2}]^{-1}$ (where Z is the number of valance electrons and C is the BGE concentration). The direction and magnitude of the EOF depends on the magnitude and the sign of the ξ potential, which itself depends on the chemical nature of the capillary surface and the BGE used. During a separation under forward polarity mode (where the positive end of the high voltage source is applied to the inlet electrode, and the negative end is applied to the outlet electrode), uncharged molecules are not affected by the electric field and have the same velocity as the EOF. Cations are attracted toward the cathode (outlet) while anions are attracted toward the anode (inlet). Since the EOF is usually several times faster than the electrophoretic mobilities of ionic analytes, all molecules move towards the outlet of the capillary; however, cations migrate faster than the EOF while anions migrate slower than the EOF. Experimentally ν_{eo} is measured by dividing the length of the capillary (from inlet to the detector) by the migration time of a neutral compound. With a known ν_{eo}, ν_{ep} can be calculated by subtracting (or adding) the contribution of ν_{eo} from the apparent (measured) electrophoretic velocity ($\nu_{ep}-\nu_{app}\pm\nu_{eo}$).

EOF plays an important role in the stable operation of electrospray ionization (ESI) [4], the most commonly used ionization technique for interfacing CE-to-MS. According to Eq. 2, factors affecting EOF include: the charge density on the capillary wall, the ionic buffer strength, the viscosity of the solution adjacent to the capillary wall, the dielectric constant of the running buffer, and the applied potential. In addition to the nature of the capillary wall, the pH of the buffer affects the degree of ionization and the polarity of the charge on the wall. Decreasing the buffer's ionic strength increases the zeta potential causing the EOF to increase. Diluting the buffer concentration decreases its ionic strength. The dielectric constant of the running buffer can be decreased by the addition of an organic solvent. EOF can also be altered by changing the viscosity of the buffer through the addition of soluble polymers (if buffer modification is used for controlling EOF; however, any compatibility issues with the specific ionization technique employed must be taken into account). These factors, in addition to surface modification of the CE capillary inner wall, can be used to optimize separation for a specific experiment [5, 6, 7, 8, 9, 10, 11].

Surface wall modification

To control EOF and reduce analyte/wall interactions, the inner wall of a fused silica capillary can be modified with either a coating covalently bound to the silanol groups on the wall surface, or by adsorption of the modifier followed by immobilization. Surface modifiers are permanently linked to the capillary wall via covalent bonds in several coating methods. For CE/ESI-MS analysis of proteins and peptides, maximal performance is usually achieved under positive ESI mode, using an aqueous acidic buffer (pH ~3.5). At these pHs, most proteins and peptides have a net positive charge, while the inner wall of the bare fused capillary is negatively charged. Derivatization of the capillary inner wall by a compound that is positively charged at this pH range reduces protein/wall interactions and maintains adequate EOF. 3-Aminopropyltrimethoxysilane (APS) de-

rivatization is commonly used to reduce analyte-wall interactions, generate a high EOF, and therefore provide stable electrospray (ES) currents as well as short migration times [12, 13]. When APS-coated capillaries are used, the CE is usually operated under reverse polarity mode to generate EOF toward the outlet. The EOF of an APS derivatized column may be too high for sufficient separation among a large number of peptides in complex mixtures, in which case the use of a polybrene-coated capillary is suggested [14, 15, 16]. Other disadvantages of APS derivatization include long derivatization times (16 h), and the possibility of plugging the capillary during the derivatization process [14].

If EOF is undesirable, the CE capillary can be coated with a monomolecular layer of non-cross-linked polyacrylamide [17]. Methylcellulose can also be used as a polymeric wall coating [18]. Non-EOF generating derivatization is usually used for capillary isoelectric focusing (CIEF) studies. Since EOF affects the residence times of solutes in the capillary, both separation efficiency and resolution are related to the magnitude of EOF [19].

Why separate?

While mass spectrometers can detect components of a mixture without prior separation, separation before mass analysis confers several advantages:

-1. Analytes are separated from salts and other impurities that can completely or partially suppress the signal of interest under ESI.
-2. Analytes can be concentrated, significantly lowering the detection limit.
-3. Component identification of the mixture is simplified.
-4. Accurate quantification is achieved through integration of separated peaks.
-5. "MS/MS" capability (via in-source fragmentation) can be utilized by mass spectrometers that have no true MS/MS capability.

These practical advantages make it desirable to separate the components of a mixture with high separation efficiency and resolution prior to mass analysis.

When diffusion is the only source of band broadening, the efficiency of the electrophoretic system (expressed as the number of theoretical plates, *N*) is given by:

$$\mathrm{N} = \mu \mathrm{V}/2\mathrm{D} \tag{3}$$

where μ is the apparent mobility and *D* is the diffusion coefficient of the individual solute. Resolution, the degree of separation between two adjacent peaks, is calculated by dividing the difference in migration distances of the peaks of interest by the average peak widths of the peaks at the baseline.

$$\text{Resolution} = 2(\mathrm{t_{slow}} - \mathrm{t_{fast}})/(\mathrm{w_1}+\mathrm{w_2}) \tag{4}$$

for adjacent peaks w_1=w_2. For Gaussian curves, the two peaks touch at the baseline when Δt=w_2; therefore, the resolution equals 1.

Factors that influence separation efficiency and resolution in CE-MS include analyte diffusion, Joule heating, injection plug width, analyte wall interaction, and the detection process. Analyte diffusion is a phenomenon common to all separation processes. Samples are usually injected into a separation capillary as a sharp concentrated solute zone. With time, the sharp concentration sample plug is broadened by diffusion to a Gaussian concentration profile. Short capillaries and high separation voltages reduce the separation time and, therefore, the diffusion of the analyte. Higher voltages, however, cause reduced resolution by enhancing analyte diffusion due to increasing the CE current and increased heat formation in the capillary. The ratio of applied potential to heat formation is:

$$\begin{aligned}\text{Appliedpotential}/\text{Heatgenerated} &= (\mathrm{i}/\kappa\pi\mathrm{r}^2)/(\mathrm{i}^2/\kappa(\pi\mathrm{r}^2)^2) \\ &= \pi r^2/i \end{aligned} \tag{5}$$

where, *i*=current, πr^2=cross section of the capillary, and κ=specific conductance of the BGE. A low conductance BGE (such as 0.1% or 0.01% acetic acid) reduces current and, along with narrow (<30 μm i.d.) capillaries that can dissipate heat effectively, allows for the use of higher electric fields, thereby providing for faster (higher throughput) and more efficient separations. An efficient separation generates narrow peaks, which translates into lower detection limits.

The width of the injection plug also affects separation efficiency and resolution. To provide good separation, prevent overloading, and avoid peak shape degradation, the length of the sample band is usually ~1% of the total capillary length. Several modes of sample introduction have been used in CE-MS analysis. The pressure (hydrodynamic) mode, in which pressure is applied to the CE inlet vial (or vacuum is applied to the outlet vial) while the CE capillary inlet is immersed in the sample vial, is most common. Because of the parabolic profile of the pressure-driven flow, however, the boundaries of the injection plug are not well defined, yielding a negative effect on separation efficiency. Another common mode of sample injection is voltage injection (electrohydrodynamic injection mode), in which the inlet end of a capillary is positioned inside the sample vial and high voltage is applied across the capillary. In this condition, analytes enter the capillary based on their electrophoretic mobilities and by the action of EOF. The plug profile of EOF ensures well-defined sample plug boundaries, but injection quantity for each analyte depends on individual electrophoretic mobility. Gravity injection is yet another common mode of sample introduction, in which the CE inlet is positioned inside the sample vial, and the vial is raised up a few centimeters above the outlet vial. Several other modes of sample injection have been reported for sampling whole cells [20]. In all cases mentioned above, the use of narrower capillaries minimizes dilution and allows for the sampling of smaller volumes. For example, injecting 1% of the total capillary length of a 50-cm, 10-μm-i.d. capillary introduces ~400 pl of sample into the capillary. Injection with minimum volume, minimum dilution, and without signif-

icant zone broadening is especially important for single cell analysis, where one attempts to introduce chemicals from a specific site of a single cell, or would like to minimize the dilution of the cell contents in whole cell sampling. While analyte/wall interaction can significantly affect separation efficiency, this interaction can be minimized by applying the proper surface modification technique, as discussed above.

The detection process, which includes the CE-to-MS interface, ESI tip, and mass spectrometer, is another factor affecting separation efficiency and resolution, and can be degraded by a number of problems:

-1. Solvent evaporation at the ESI tip. This induces a hydrodynamic flow inside the column [21] and degrades the flat flow profile of EOF.
-2. Bubble formation due to electrochemical reactions at the CE outlet/ESI electrode. This causes fluctuation of the ES and an uneven accumulation of liquid at the ESI tip [22].
-3. Peak broadening due to slow data acquisition. This occurs if the data acquisition rate of the MS is not compatible with the CE peak width.
-4. A CE-MS interface design that diminishes the resolution of CE/ESI-MS.

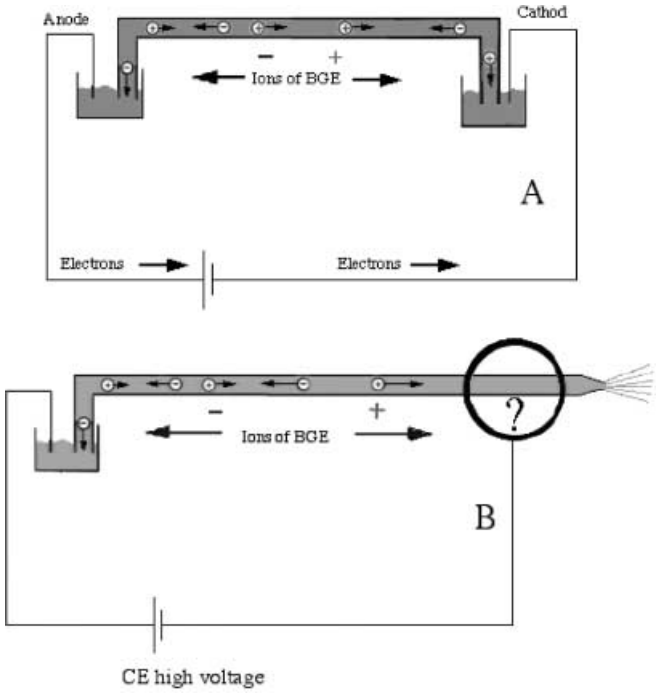

Fig. 1 **A** The general schematic of capillary electrophoresis (*CE*). **B** On-line CE-MS where the outlet end of the CE is removed from outlet vial and is positioned in front of the mass spectrometer

CE-to-MS interface

The general schematic of CE is shown in Fig. 1A. Upon application of high voltage, anions in the BGE solution migrate toward the anode (positive electrode), while cations migrate toward the cathode (negative electrode) in equal quantities. The electroneutrality of the solution is maintained by redox reactions of water at the electrodes. The CE, therefore, acts as a complete electrical circuit, in which ions move in the solution while electrons move in the wire connecting the power supply to the CE inlet/outlet electrodes. For on-line CE-MS, the outlet end of the CE must be removed from the outlet vial and positioned in front of the mass spectrometer inlet (Fig. 1B). This process, however, opens the CE electrical circuit and terminates CE operation unless a voltage connection is provided at the CE outlet. Under ESI, the connection that serves to close the CE electrical circuit also provides a connection for the ESI voltage. The method used to establish and maintain this electrical connection is a crucial feature of any CE-MS interface [23, 24]. In addition to robustness and ease of fabrication, other important features of all CE-MS interfaces include the following.

-1. The interface should maintain the resolution of the CE. Narrow peaks generated by CE are usually a few seconds wide or less. Considering that the buffer flow of narrow CE capillaries is in the range of 100–200 nl/min, any dead volume in the interface area will significantly degrade CE separation, and therefore, the detection limit.
-2. The interface should provide the highest sensitivity for analyte detection. An important feature of ESI is that (at flow rates of >~100 nl/min [23]) it is a concentration-sensitive ionization technique and, therefore, the dilution of analyte by liquids at the interface area will decrease the sensitivity of detection.
-3. The interface must be able to accommodate narrow capillaries. Similar to nanoelectrospray [25, 26], ESI generated from narrow capillaries produces smaller droplets and improved sensitivity [21, 27, 28, 29, 30].
-4. Because of their low-flow and narrow tips, narrow capillaries can be positioned very close to the MS inlet for maximum ion transport to the MS, further increasing the sensitivity of detection.

Over the past 15 years, a variety of CE/ESI-MS interfaces have been introduced. These interfaces divide into two general categories: sheath-flow interfaces and sheathless interfaces [23, 24, 31, 32].

Sheath-flow interfaces

In the sheath-flow configuration, the electrical connection to the CE outlet is achieved with a sheath liquid that mixes with the CE buffer at the CE outlet through coaxial tubing [33] (Fig. 2A), or enters an ESI-emitter [34] or nanospray tip [35] via a liquid junction (Fig. 2B). Sheath-flow configurations with coaxial tubing, in which the outlet of the CE capillary is simply inserted into the ESI emitter (a piece of stainless steel tubing, commonly referred to as the ESI needle), have several advantages, including simple fabrication, reliability, and ease of implementation. These advantages make them the most widely used inter-

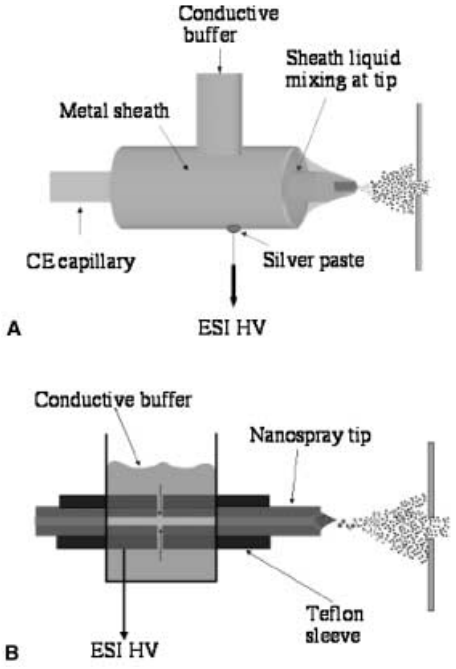

Fig. 2 **A** A liquid sheath interface in which CE buffer mixes with a sheath solution at the CE outlet to establish electrical connection. **B** A liquid junction in which sheath liquid is mixed with CE buffer before it reaches the electrospray ionization (*ESI*) tip

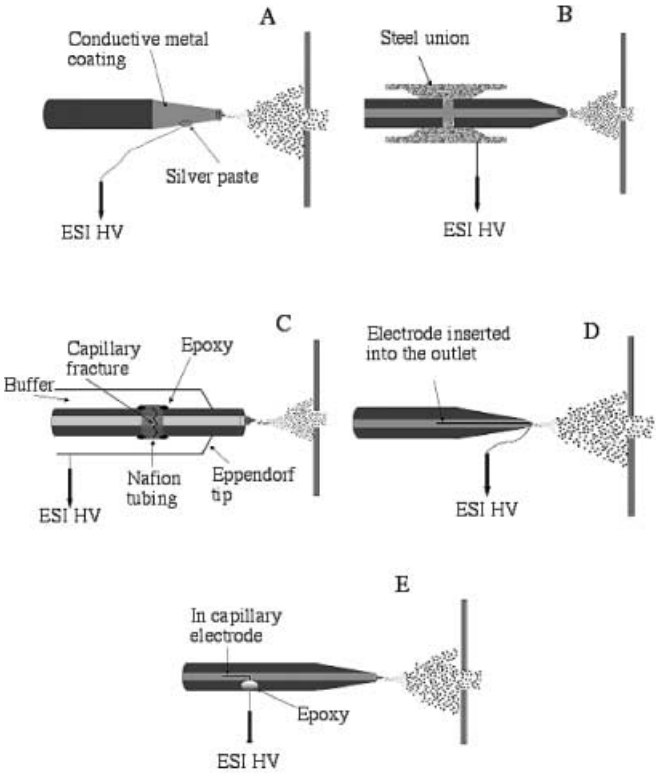

Fig. 3A–E Schematics of several sheathless interfaces. **A** A metal-coated tip interface. **B** A low dead volume (stainless steel) union interface. **C** A low dead volume union using Nafion tubing. **D** A sheathless interface using a wire inserted into the CE capillary outlet. **E** In-capillary sheathless interface where a wire is inserted into a small opening near the CE outlet and sealed in place with epoxy

faces for routing CE/ESI-MS analysis. The main disadvantage of sheath-flow configurations, however, is their low sensitivity of detection due to the dilution of the analyte by the sheath liquid.

Sheathless interfaces

In sheathless interfaces, the electrical connection to the capillary outlet is achieved by direct metal/liquid contact at or near the CE capillary outlet [36, 37, 38, 39, 40, 41, 42, 43, 44, 45]. A variety of sheathless interfaces have been introduced:

-1. CE with a metal-coated [38] (Fig. 3A), or gold particle-coated ESI tip [43]. The major disadvantage of the metal coated tip is the short lifetime of the coating, due to electrochemical/electrical degradation under CE/ESI-MS.
-2. Attaching a nanospray tip to the CE outlet with a low dead volume (stainless steel) union [42] (Fig. 3B) or with a piece of Nafion tubing (Fig. 3C) [35, 36, 37, 38, 39, 40, 41, 42, 43, 44]. Because the capillary inner diameter is usually smaller than the wall thickness, the major disadvantage of this technique is the significant dead volume where the two capillaries are joined.
-3. Insertion of a wire into the CE capillary outlet [39, 40, 41, 42, 43, 44, 45] (Fig. 3D) or through a small opening near the CE outlet (Fig. 3E), where it is sealed in place using epoxy [46, 47, 48].

Because there is no sheath liquid present to dilute the CE effluent, the major advantage of the sheathless interface is its high sensitivity. Figure 4 demonstrates the sensitivity advantages of sheathless CE-MS with a buffer flow rate of ~200 nl/min compared to a sheath liquid interface with a similar buffer flow rate and a sheath liquid flow rate of ~2 µl/min. The amount of sample that was injected in the sheath flow column was 240 times more than the amount injected into the sheathless column. The signal intensity of the sheathless column's sample, however, was lower by only a factor of 3. Figure 5A and B demonstrate the sensitivity advantages of a sheathless CE-MS with a buffer flow rate of ~500 nl/min compared to the µ-HPLC-MS analysis of the same sample using a 50 µl/min flow rate using the Sigma HPLC peptide standard. While only 1/1000 of the sample was injected into the CE capillary (~5 nl vs. 5 µl injected into the HPLC), the sensitivity response of the CE/ESI-MS was very similar to that of the HPLC/ESI-MS on the absolute scale. However, because pneumatically assisted ESI at 50 µl/min under HPLC-MS is more stable than conventional (pneumatically unassisted) ESI at low flow rates under sheathless CE-MS, the background with the latter interface was noisier. The results show that because of the concentration-sensitive nature of ESI, there is a clear sensitivity advantage in using low flow rate techniques such as sheathless CE-MS. Other advantages of CE over capillary LC include protein analysis capability and the ability to introduce very small sample sizes, such as single cells (see below).

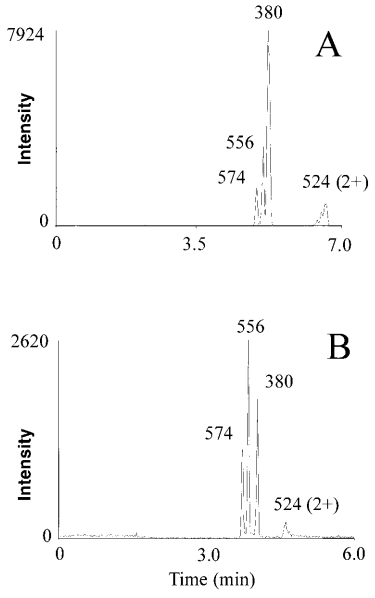

Fig. 4A, B Demonstrating the sensitivity advantage of **B** sheathless (using an in-capillary interface) CE-MS vs. **A** sheath-flow (liquid sheath interface) CE-MS. While the sample injected in the sheathless experiment was 100 times more dilute than in the sheath-flow experiment, the signal intensity of the sheathless interface was lower by only a factor of 3. A 50-µm-i.d., ~50-cm-long, 3-aminopropyltrimethoxysilane (APS) derivatized, in-capillary electrode was used for the CE-MS study. Sigma HPLC peptide standard was used. The TOF MS was scanned in the *m/z* of 375–600. Peaks are marked with their corresponding *m/z* and are respectively methionine-enkaphalin (MW 573), leucine-enkaphalin (MW 555), Val-Tyr-Val (MW 379), and angiotensin II (MW 1046)

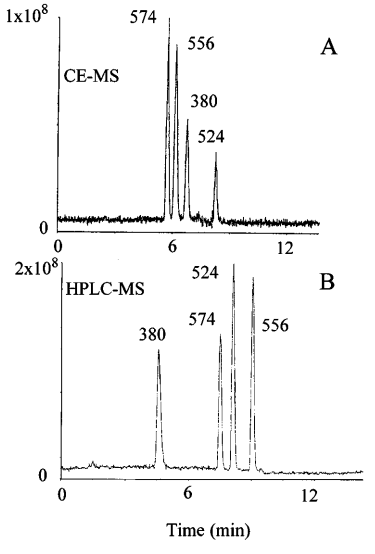

Fig. 5A, B Sensitivity comparison between **A** sheathless CE-MS and **B** capillary HPLC-MS. A 50-µm-i.d., ~50-cm-long, APS derivatized, in-capillary electrode was used for the CE-MS study with a buffer flow rate of ~500 nl/min. A 500-µm-i.d., C18 column with a flow rate of 50 µl/min was used with the HPLC. Mobile phase was water+acetonitrile+0.1% TFA. Only 1/1000 of the sample was injected into the CE capillary (~ 5 nl vs. 5 µl injected into the HPLC). See caption of Fig. 4 for peak identification

While wire designs are robust and there is no dead volume associated with them, their application is limited to capillaries with inner diameters >25 µm. Another disadvantage of the wire design is the formation of bubbles inside the capillary due to electrochemical water redox reactions that occur at the electrode, as discussed below.

CE as an electrochemical cell

CE separation requires the application of high voltage to electrode(s) within a running buffer (Fig. 1). Hence, each electrode within the buffer forms a half-cell at which electrochemical reactions take place. In most cases, inert metals such as platinum (Pt) are used as electrodes. Since an aqueous solution is commonly used as the running buffer, in the absence of any compound with a redox potential below that of water, the major reactions at these electrodes are oxidation (at the anode) and reduction (at the cathode) of water, according to the following half-reactions (at pH=7) [49]:

$$\begin{aligned} &O_2\,(g) + 4H^+ + 4e^- \leftrightarrow 2H_2O \\ &E^o_{red} = +0.816\ V\ \ (\text{vs. SHE}) \end{aligned} \tag{6}$$

$$\begin{aligned} &2H_2O + 2e^- \leftrightarrow H_2\,(g) + 2OH^- \\ &E^o_{red} = -0.413\ V\ (\text{vs. SHE}) \end{aligned} \tag{7}$$

The extent of these reactions depends on the electrophoretic current (i_{CE}) inside the capillary, generated by the movement of charged species from the BGE under the action of an electric field. The current is controlled by several factors, including the cross section of the column (*S*), the magnitude of the electrical field (*E*), and the conductivity (*k*) of the electrolyte [2].

$$i_{CE} = SEk = SEF \sum\nolimits_j z_j \hat{u}_j c_j \tag{8}$$

Here, z_j is the charge of component j, $\hat{u}_j$ is the effective mobility of j, and c_j is its concentration. In the CE capillary, cations and anions of the BGE move in opposite directions, so the total current (i_{CE}) is the sum of all of the ion currents. Under a specific set of experimental conditions (constant temperature, concentration of BGE, capillary diameter, and separation voltage), the CE current is fixed. Thus, the CE acts as a constant-current electrolytic cell. Since only electrons can move through the external wire that supplies potential to the electrodes, oxidation and reduction proceed at the anode and cathode respectively, to maintain the electroneutrality of the cell. As a result of reactions shown by Eq. 6 and Eq. 7, the pH of the solution decreases at the anode and increases at the cathode, and bubbles are formed at both electrodes due to the production of gas. For Eq. 6 and Eq. 7, the concentration of the ionic species and the volume of gas produced at each electrode can be calculated by Faraday's law [50]:

$$\left[H^+\right] = i_{CE}/nF\nu_f \tag{9}$$

$$Q = i_{CE}\Delta t = nFx \tag{10}$$

where i_{CE} is the CE current, n is the number of electrons participating in the redox reaction at the electrode, F is the Faraday constant (96485 C/mol e^-), ν_f is the CE flow rate, and x is the number of moles of gas produced per mole of electrons transferred at the electrode.

Under conventional CE, since the electrodes are positioned away from the ends of the separation capillary, bubbles formed at the electrodes have minimal effect on the separation efficiency. However, the pH change of the separation buffer has been shown to have a significant effect on the reproducibility of analysis [51, 52, 53, 54], yet its effect can be minimized by the frequent replacement of the buffer solution. Bubble formation, however, can have a significant negative impact on the performance of the CE-MS analysis, especially at low CE buffer flow rates. One way to reduce the negative effects of water electrolysis is to reduce the CE current. Among factors that reduce i_{CE} are the use of a BGE with low ionic conductance, use of a low concentration BGE, and the use of narrow capillaries.

ESI as an electrochemical cell

The electrolytic nature of ES has been well studied (see reference [4], chapters 1 and 2, and references therein). Application of high voltage (2–5 kV in positive ionization mode, the only ionization mode discussed here) to a conductive solution exiting a capillary pointed toward a counter electrode (such as the MS inlet) at low potential (0–200 V) initiates the formation of a Taylor cone at the tip of the capillary, which is enriched with positive electrolyte ions. Excess positive charge is due to electrophoretic separation of positive and negative ions at the ES electrode and electrochemical oxidation of water at this electrode (anode), which pumps an excessive quantity of protons into the solution. Emission of positively charged droplets from the tip of the Taylor cone and solvent evaporation from the charged droplets leads to the formation of positively charged ions. Therefore, the ESI needle and its counter electrode act as an electrolytic cell of special kind because flow of charge to one of the electrodes (MS inlet) is achieved through the air rather than through a buffer solution [55]. The ES current (i_{ES}) is given by:

$$i_{ES} = A_H V^{\nu} E^{\varepsilon} \sigma^{n} \tag{11}$$

where A_H is a constant and depends on the dielectric constant and surface tension of the solvent, V^{ν} is the buffer flow rate, E^{ε} is the electric field at the cone tip, and σ^{n} is the conductance of solution. The value of individual exponents may vary according to the experimental parameters. As shown in Eq. 11), the i_{ES} depends on several factors including the solution conductivity and the magnitude of the electric field at the tip of the ES emitter [55].

As was the case for CE, in the absence of any easily oxidized substance (including the electrode that supplies voltage to the ES unit and has contact with electrolyte), the reactions in Eq. 6 and Eq. 7 will proceed to the extent dictated by the ES current. The consequences of these electrochemical reactions include degradation of the ES electrode, pH reduction at the tip of the ES needle, and fluctuation of the ES plume due to bubble formation.

Another important consequence of the electrochemical nature of ESI is oxidation of the analyte at low flow rates under high ESI voltage. As shown in Eq. 11, the i_{ES} is proportional to the ESI voltage. Under very high current densities (high ESI voltage) and low buffer flow rates, where the redox reaction (Eq. 6) at the anode is unable to supply the current generated at the ESI electrode, electrolysis reactions of water with higher redox potentials (reactions shown in Eq. 12 and Eq. 13) will occur to supply the necessary current [56].

$$\begin{aligned} &H_2O_2 + 2H^+ + 2e^- \leftrightarrow 2H_2O \\ &E^o_{red} = 1.776 \text{ V (vs. SHE)} \end{aligned} \tag{12}$$

$$\begin{aligned} &O\,(g) + 2H^+ + 2e^- \leftrightarrow H_2O \\ &E^o_{red} = -2.42 \text{ V (vs. SHE)} \end{aligned} \tag{13}$$

Interactions of reactive species generated in these reactions with peptides are proposed to be most responsible for the oxidation of peptides at low flow rates. Oxidation of analytes significantly reduces the sensitivity of detection by diluting the analyte signal over several oxide species [57]. The extent of these reactions depends on i_{ES}, which itself depends on the electric field at the ES tip. Since it is the geometry of the tip (and therefore the electric field at the CE outlet/ESI tip) that dictates the voltage necessary for ESI operation [55], sharpening the capillary outlet (by HF etching, for example) can significantly enhance sensitivity by reducing ESI voltage, ESI current, and peptide oxidation. In order to minimize oxidation, it is important to set the ESI voltage very close to the minimum voltage required to maintain stable ES (V_{on}). Theoretically, the voltage required to initiate charged droplet emission (the onset of ES) from the ES tip is given by:

$$V_{ON} \approx 2 \times 10^5 \, (\gamma r_c)^{1/2} \ln (4d/r_c) \tag{14}$$

where γ is the surface tension of the buffer (for water γ= 0.073 N/m^2), r_c is the capillary radius at the tip (1/2 of the capillary outer diameter at the tip), and d is the distance between capillary tip and counter electrode (MS inlet). For example, for a capillary radius of 20 μm, positioned 2 mm away from the MS inlet, $V_{ON} \approx 2\times10^5 \, (0.073\times20\times10^{-6})^{1/2} \ln (4\times2\text{x}10^{-3}/20\times10^{-6}) \approx 1450$ V. Experimentally we have found that at the low flow rates of narrow capillaries, peptide oxidation is very sensitive to the ES voltage, and peptide oxidation can be observed at even 100 V above the threshold. Therefore, there is a narrow window between stable ES operation and peptide oxidation.

Electrochemical phenomena explained above for CE and ESI processes are exacerbated under CE/ESI-MS [58]. As shown in Fig. 6, CE/ESI-MS overlays two controlled current techniques to form a three-electrode system (CE inlet, shared CE outlet/ES emitter, and MS inlet electrodes), in which one electrode is shared between CE and ESI circuits. Negative effects of these electrochemical reactions under sheathless CE/ESI-MS include: (1) the formation of bubbles, which degrades resolution, (2) the ox-

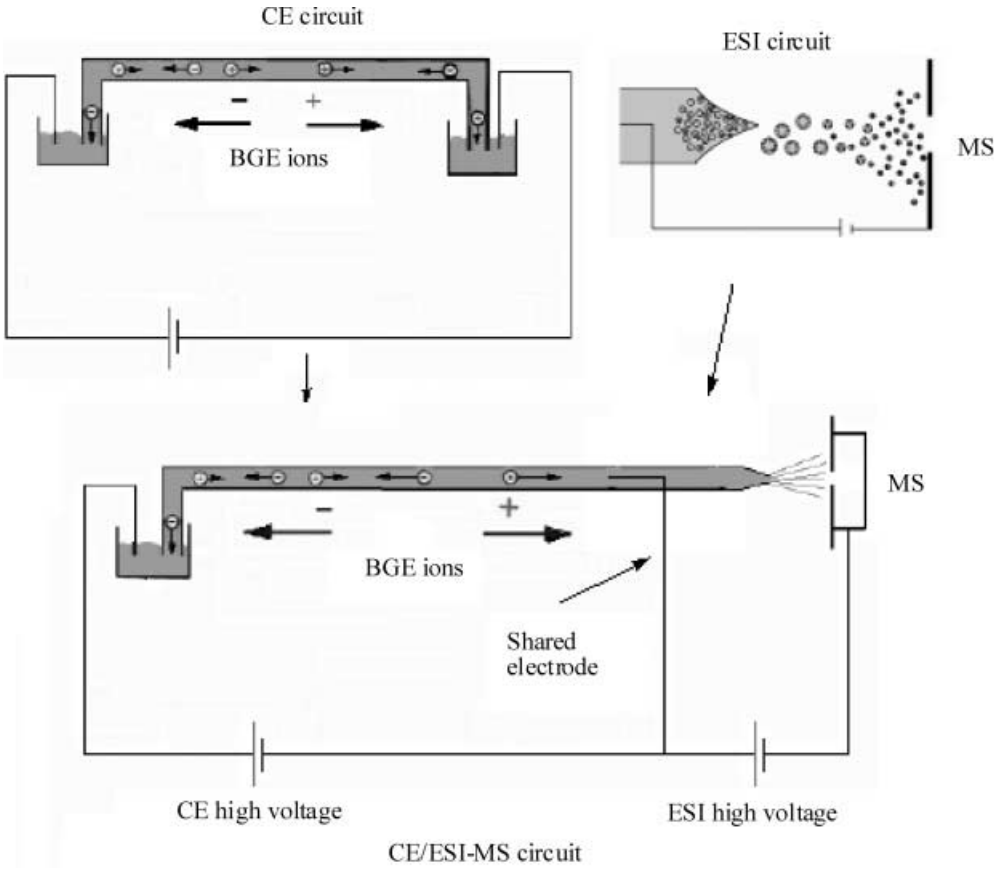

Fig. 6 Schematic of a sheathless CE/ESI-MS in which two controlled current techniques CE and ESI are overlaid, with one shared electrode (CE outlet/ESI electrode)

idation of analytes, which degrades sensitivity, and (3) the degradation of reactive electrodes, which shortens the lifetime of the interface. In addition to the use of narrow capillaries, buffers with low ionic conductance and sharp ES tips (which reduce i_{CE} and i_{ES}, respectively) can minimize these effects. The electrochemical reactions mentioned above, however, can be controlled by the use of redox buffers and/or by an interface design that can minimize these effects.

Control of the CE and ESI electrochemical reactions

The function of the redox buffer is to hold the potential at the shared electrode below that necessary to oxidize and/or reduce water, and thereby eliminate bubble formation due to electrolysis. In general, two types of redox buffers have been used [59]. One type uses a reactive metal electrode (such as iron or zinc) as the anodic "redox buffer." In this case, oxidation (corrosion) of the metal electrode and formation of metal ions replaces electrochemical oxidation of water. The advantage of using a reactive metal electrode is that it not only suppresses bubble formation and analyte oxidation, but it also maintains the pH of the solution. Another type of redox buffer is a reactive compound with a redox potential below that of water, which can be added to the CE and/or ESI running buffer. For example, *p*-benzoquinone might be used as a cathodic buffer and hydroquinone might be used as an anodic buffer.

Split-flow interface

To address the bubble problem, and to simplify the CE-to-MS interfacing specifically for narrow i.d. capillaries, we recently introduced a split-flow CE/ESI-MS interface (Fig. 7), in which the electrical connection to the CE capillary/ESI electrode was achieved by diverting part of the CE buffer out of the capillary through an opening near the capillary outlet [60]. The CE buffer exiting the opening contacts a metal sheath tube (or ESI needle) which acts as the CE outlet/ESI shared electrode. Due to the concentration sensitive nature of ESI, splitting a small percentage of the CE flow has a minimal effect on the sensitivity of detection. In addition, because liquid is flowing through the opening and out of the capillary, there is no dead vol-

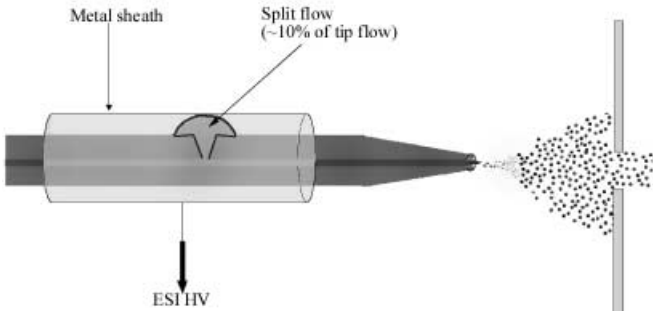

Fig. 7 Schematic of the split-flow CE/ESI-MS interface

ume associated with this interface. Moreover, bubble formation (due to redox reactions of water at the electrode) does not affect CE/ESI-MS performance, since actual metal/liquid contact occurs outside of the CE capillary.

Types of mass spectrometers used in CE-ESI-MS

Almost all kinds of mass spectrometers have been used for CE/ESI-MS analysis. Most CE/MS analyses to date, however, have used mass spectrometers that are capable of operating with only unit mass resolution and low mass accuracy. This is because until recently only magnetic sector [61, 62] and Fourier transform ion cyclotron resonance (FTICR)[63] mass spectrometers were capable of generating high resolution, high mass accuracy mass spectra under ESI. High resolution, high mass accuracy operation of these instruments, however, usually requires long acquisition times that are incompatible with the narrow peak widths generated by CE [64]. The new generation of FTICR with high magnetic fields (>9 Tesla) is capable of attomole and sub-attomole sensitivity at very high resolutions (>100,000 for ESI) and very high mass accuracy (<1 ppm) at ~1 s acquisition times [65]. These characteristics make the modern FTICR MS an ideal detector for most CE analysis. However, the high cost of FTICR mass spectrometers keeps them out of reach for most mass spectrometrists. Recent advances in time of flight (TOF) MS have made it possible to achieve subfemtomole detection limits, with a resolution of ~5000, a mass accuracy of <10 ppm, and fast acquisition rates (0.1 s), all at a fraction of the cost of the FTICR MS [66, 67, 68, 69, 70, 71, 72]. These characteristics make TOF MS an ideal detector for capillary electrophoresis. At this time, the detection limit of TOF MS is at mid attomole levels [73], and is therefore not suitable for more demanding applications, such as the analysis of the chemical contents of a single cell, in which the target may be present at low attomole or sub attomole levels. More recently, we have been able to achieve low attomole detection limits by using an ion trap mass spectrometer [74].

Unless otherwise mentioned, the equipment used throughout the manuscript includes a Mariner (Perseptive Biosystems, Framingham, Mass.) TOF MS, an MDQ (Beckman Instrument, Fullerton, Calif.) operated under reverse polarity mode at –30 kV, and a Magic 2002 HPLC (Michrom Bioresources, Auburn, Calif.). Unless otherwise mentioned, the buffer used for all CE-MS experiments was 0.1% acetic acid in water (pH 3.5). See respective publications for experimental details.

Applications to biological mixtures

To date, while efforts in CE-MS have mostly concentrated on the development of CE-to-MS interface designs, many studies regarding the application of CE-MS to the analysis of complex biological mixtures exist. In addition to the routine analysis of pharmaceutical drugs, the analysis of proteins and peptide mixtures has received particular attention, as has the analysis of the chemical contents of intact cells.

Protein identification using high mass accuracy peptide mapping techniques

Peptide mapping has been routinely used for protein identification using MS. CE/ESI-MS has been used for rapid identification of whole protein mixtures and protein digests. Recently, we used CE/ESI-TOF MS to characterize small proteins using peptide mapping [48]. First, intact proteins were analyzed by CE-MS to obtain their average molecular weights with an accuracy of about 0.03% (Fig. 8). For example, Fig. 8 shows the total ion electropherogram (TIE) of a protein mixture composed of β-lactoglobulin A (peak 1, MW 18363), ribonuclease A (peak 2, MW 13682), myoglobin (peak 3, MW 16951), lysozyme (peak 4, MW 14295), and cytochrome c (peak 5, MW 12360). On-line CE-MS analysis of the tryptic digests of these small proteins was then performed to obtain the accurate molecular weights of the peptides with accuracies of ~10 ppm. Figure 9A (cytochrome) and B (hemoglobin S) show typical CE/ESI-MS electropherograms of the tryptic digest of small proteins (see [22] for experimental details). Table 1 summarizes the results for the tryptic digest of cytochrome c. Next, this information was used for the identification of the proteins using a protein database. As shown in Table 2, it is clear that the high mass accuracy of the analysis effectively reduces the list of proteins generated by the database. Moreover, on-line in-source fragmentation of the completely or partially resolved peptide peaks produced accurate mass sequence information that was used to identify these peptides unambiguously. Each CE/ESI-MS analysis used ~5 nl of sample containing ~120 fmol of each peptide in protein digests. The results

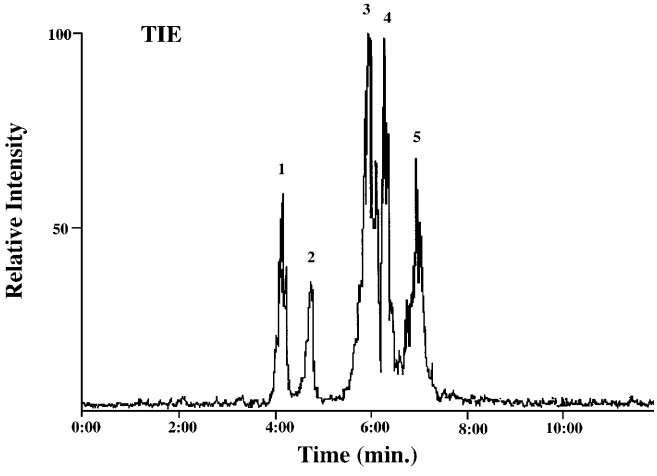

Fig. 8 Total ion electropherogram (*TIE*)of a protein mixture using the in-capillary electrode CE/ESI-MS sheathless interface. *1*, β-lactoglobulin A, *2* ribonuclease A, *3* myoglobin, *4* lysozyme, *5* cytochrome c. A 0.01 mol/l acetic acid buffer was used. The mass spectrometer utilized was a Finnigan MAT TSQ 700. Adapted from [46]

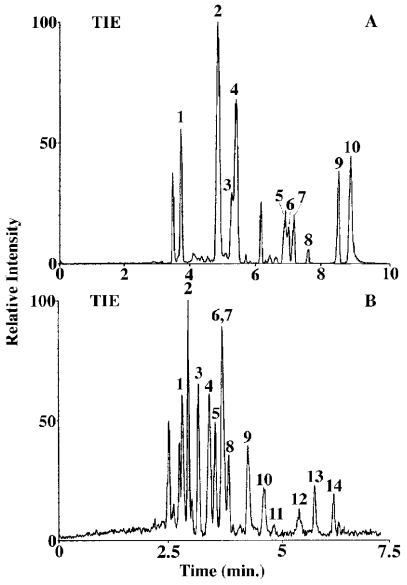

Fig. 9 **A** The TIE of CE/ESI-MS analysis of tryptic digests of cytochrome *c*. *1* Acetyl-GDVE, *2* EDLIAYLK, *3* TCQAPGFTYTDANK, *4* EETLMEYLENPK. *5* YIPGTK, *6* MIFAGIK. *7* MIFAGIK, *8* GITWK, *9* TGPNLHGLFGR, *10* CAQCHTVEK(heme). B The TIE of CE/ESI-MS analysis of tryptic digests of hemoglobin S. *1* FFESFGDLSTPDAVMGNPK, *2* VNVDEVGGEALGR, *3* EFTPPVQAAYPK, *4* FLASVSTVLTSK, *5* LLVVYPWTQR, *6* MFLSFPTTK, *7* VLGAFSDGLAHLDNLK, *8* SAVTALWGK, *9* VGAHAGEYGAEALER, *10* LHVDPENFR, *11* AAWGK, *12* VVAGVANALAHK, *13* TNVK, *14* VHLTPVEK. Unmarked peaks are not identified. Adapted from [22]

Table 1 Measured masses of tryptic digests of horse cytochrome *c* in the *m*/*z* range 500–1000. The cytochrome C sequence is: Acetyl-DVEKGKKIFVQKCAQCHTVEK(heme)GGKHKTGPNLHGLFGRKTGQAPGFTYTDANKNKGITWKEETLMEYLENPKKYIPGTKMIFAGIKKKTEREDLIAYLKKATNE (104 amino acids, calculated average MW 12359.80 Da)

AA sequence	*m*/*z* (calculated)	*m*/*z* (measured)	Charge state	Error (ppm)
Acetyl-GDVEK	589.283	589.283	+1	<1.0
EDLIAYLK	964.536	964.529	+1	7.3
TGQAPGFTYTDANK	735.847	735.857	+2	12.2
EETLMEYLENPK	748.354	748.362	+2	10.7
YIPGTK	67383	678.383	+1	<1.0
MIFAGIK	779.449	779.440	+1	11.5
IFVQK	634.385	634.387	+1	3.2
GITWK	604.346	604.343	+1	5.0
TGPNLHGLFGR	584.815	584.825	+2	17.1
CAQCHTVEK(heme)	545.217	545.223	+3	11.0

indicated that the combination of CE and high mass accuracy TOF MS is a viable option for the identification of small proteins using peptide mapping. Because of the advantages of high mass accuracy in protein identification, we recently developed a multi-ESI sprayer, multi-nozzle MS in which both reference and unknown peptides are introduced into the MS at the same time. This arrangement significantly improved the measured mass accuracy and, therefore, protein identification.

Table 2 Search parameters and numbers of protein matches for cytochrome *c* digest

Peptide mass accuracy (ppm)	1000	100	15	10
Number of peptides required for match	4	4	4	4
Number of peptides used for match in search	10	10	10	10
Number of proteins matched	>500	89	16	7

Analysis of the chemical contents of cells

There are two approaches to the mass spectrometric analysis of the chemical contents of a single intact cell: (1) direct analysis of the entire contents of the cell using laser desorption techniques (microspot matrix-assisted laser desorption/ionization-MALDI [75] or direct laser vaporization/ionization [76]), and (2) on-line separation, ionization, and detection of cell contents using CE/ESI-MS [77, 78]. The first approach has a faster analysis time, since no on-line separation is performed, but additional sample clean-ups and the transfer of cells to a new buffer system [76], or off-line sampling and lysing [75] are necessary for successful MS analysis.

For on-line separation, CZE, with its rapid and highly efficient separation of compounds in extremely small volume samples (<1 nl), has rapidly become the separation method of choice for single-cell analysis [77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88]. The overall detection limit of CE/ESI-MS depends not only on the inherent detection limit of the mass spectrometer, but also on the CE and ESI processes. The concentration-sensitive nature of ESI, along with the narrow peaks generated by CE, provides optimum sensitivity. However, sample loss due to irreversible adsorption onto the capillary wall, incomplete ionization of analyte under ESI, and ion loss during transport from the atmospheric pressure ionization region to the mass spectrometer analyzer are among other factors that can affect the overall sensitivity. Therefore, a successful CE/ESI-MS analysis of the major chemical contents of a single cell requires a highly sensitive mass spectrometer that can compensate for analyte dilution/loss during CE and ESI processes, and a highly efficient CE and CE-to-MS interface that can provide high separation and high ion transport efficiency.

At this time, on-line CE/ESI-MS analysis of intact cells is limited to human red blood cells (RBCs) [77, 78]. The RBC contains compounds in a wide quantity range and acts as a benchmark for the performance of CE-MS in the analysis of complex mixtures present in small quantities. The most abundant protein of RBCs, hemoglobin (Hb, ~450 attomoles/cell), is well within the sensitivity range of today's mass spectrometers. The α- and β-chains of hemoglobin in a single intact RBC have been detected using FTICR [77] and TOF MS [78]. Figure 10 shows separation and detection of α- and β-chains of HbA in a single intact RBC using TOF MS. Detection of carbonic anhydrase (CAI and CAII), which are the next most abundant proteins found in RBCs, at quantities of ~7 amol for CAI and ~0.8 amol for CAII in adult RBCs [89, 90], has

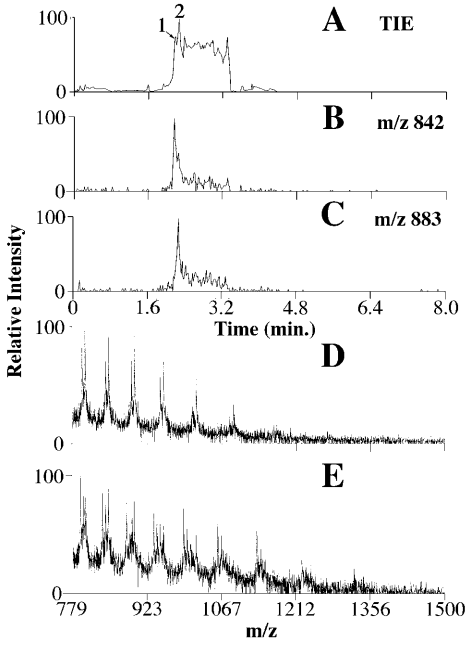

Fig. 10 **A** TIE, and reconstructed ion electropherograms with **B** *m/z* 842 and **C** *m/z* 883 corresponding to the 18^+ charge state of the α- and β-chains of hemoglobin, respectively, obtained from injection of one intact red blood cell into the CE capillary. **D** and **E** are mass spectra corresponding to the peaks of **B** and **C**, respectively. Adapted from [47]

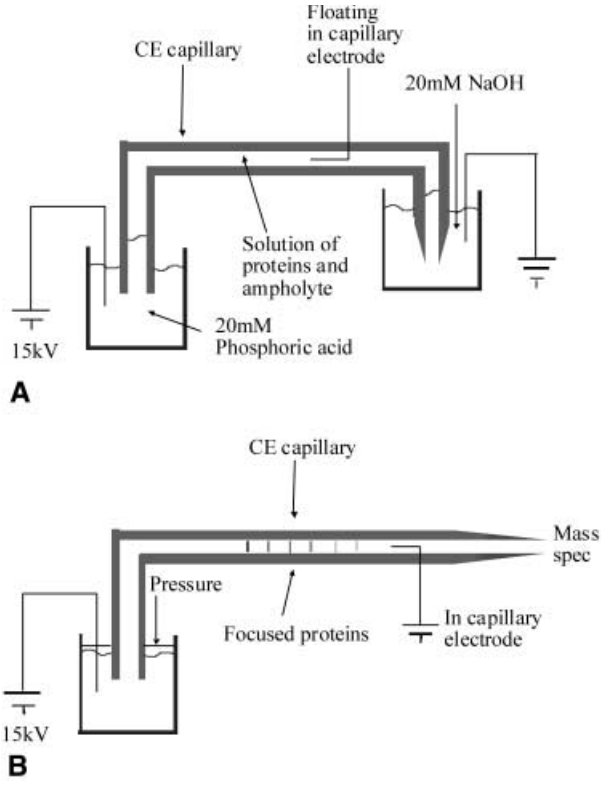

Fig. 11A, B Schematic of a sheathless capillary isoelectric focusing (CIEF) before (**A**) and after focusing (**B**)

been more challenging. In both recent studies of a single intact RBC using CE/ESI-MS, CA was not detected. However, using a crude isolate from human blood, CAI was detected by CE/ESI-MS utilizing FTICR [28]. The quest for detection of the chemical contents of cells at lower quantities has begun. For example, recently, we used CE/ESI-MS using quadrupole ion traps to investigate the chemical contents of single human RBCs at the level of ~three cells, and were able to detect CAI [74].

Sample concentration techniques in CE-MS

A drawback of conventional CE is its inefficient analysis of dilute samples. Unlike HPLC, standard CE does not focus dilute samples. Introducing more sample only diminishes resolution. Considering that the volume of narrow CE capillaries is less than ~1 μl, only finite volumes (<10 nl) of sample can be injected. To remedy the high concentration detection limit of CE, several focusing techniques have been used, including isoelectric focusing, on-column transient isotachophoresis, membrane concentrators, and packing the inlet of the CE capillary with C_{18} (or other affinity packing material).

CIEF/ESI-MS analysis

In CIEF, a pH gradient is achieved by filling the entire capillary with dilute samples of proteins dissolved in a 0.5–1% solution of ampholytes (a series of zwitterionic species) and placing one end of the capillary in an acidic solution (such as 20 mM phosphoric acid or acetic acid) [91, 92] and the other end in an alkaline solution (such as 20 mM sodium hydroxide or ammonium acetate). The acidic solution acts as the anode, and the alkaline solution acts as the cathode. Once the voltage is applied, a pH gradient is formed inside the capillary, and positively charged proteins migrate toward the cathode, while negatively charged proteins migrate toward the anode. As the proteins approach their isoelectric point (pI), they gradually become less and less charged until they have a net charge of zero and, therefore, zero mobility. The proteins are thus concentrated in a narrow zone. Any protein molecule diffusing away from its pI zone will acquire a net charge and be transferred back to its pI by electrophoresis. The proteins are therefore focused corresponding to their own pIs. This method can be used to stack large volumes of proteins from dilute solutions. Once the focusing has finished, the contents of the capillary are mobilized toward the detector, typically by voltage, pressure, gravity mobilization, or some combination of the three. In addition to providing MW information, CIEF MS provides pI information for proteins. The capillary is usually derivatized using a polymeric coating to prevent EOF. The most common polymers used for capillary wall coating are methylcellulose and polyacrylamide [17, 18].

CIEF analysis of a large number of proteins with a wide range of pIs can be performed with an ampholyte under a wide pH range, such as pH 3–10. As an alterna-

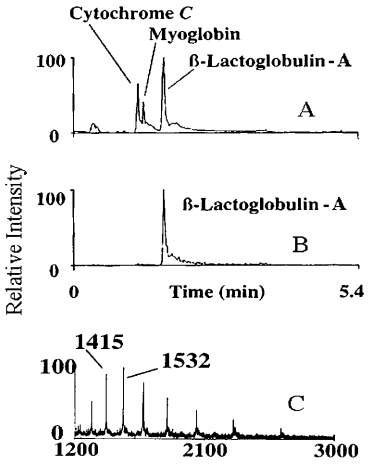

Fig. 12A–C TIE of a mixture of three proteins using a sheathless CIEF (**A**), an ion electropherogram of β-lactoglobulin (**B**), and its mass spectrum (**C**). Adapted from [120]

tive and/or complementary technique to two dimensional gel electrophoresis, Smith and co-workers recently used CIEF-FTICR MS in conjunction with isotopically labeled amino acids to identify intact proteins on a proteome wide level [92]. The use of an ampholyte within a narrow range, such as pH 6–8 allows for higher resolution, as is the case in hemoglobin analysis [93]. Two important factors for successful CIEF analysis include elimination of BGE migration during focusing, and proper mobilization of the focused analyte zones for detection. Sensitivity is still another issue with MS, since the existence of ampholytes tends to suppress the protein signals under ESI MS. In addition, the base can suppress the signal of the very basic proteins that are focused near the cathode. Figure 11A, B shows the schematic of a sheathless CIEF before and after focusing, respectively. Figure 12A, B, and C shows, respectively, the TIE of a mixture of three proteins using a sheathless CIEF, an ion electropherogram of β-lactoglobulin, and its mass spectrum.

On-column transient isotachophoresis CE/ESI MS

Yet another technique that has been used to enhance the concentration detection limit of CE/ESI-MS is isotachophoresis (ITP). In ITP the sample zone is sandwiched between a leading and a terminating buffer. During the separation, both the leading and terminating zones are separated from the sample by a phase boundary and analytes are focused based on the molarity of the leading buffer. The direct coupling of capillary ITP (CITP) to MS [94], or CITP as a preconcentration step for CE-MS using either two separate columns [95] or one column (where it is called on-column transient ITP), has been applied to a variety of compounds [96, 97]. More recently, on-line coupling of protein digestion with transient CITP/CZE-ESI-MS as an integrated microfluidic system has been used for on-line protein digestion and peptide separation [98].

Membrane preconcentration

Another technique for on-line preconcentration prior to separation is the use of a short, semi-permeable hollow fiber connected to the inlet end of the CE capillary [99]. An injection electric field is applied across the hollow fiber from the sample vial. Since proteins cannot pass through the membrane, they are concentrated at the inner wall of the membrane. After a specific period of injection (concentration) time, the CE separation is initiated. By using this technique a 1000-fold lower detection has been achieved for proteins. This technique, however, is not applicable to compounds with low molecular weight since they pass through the hollow fiber.

A polymeric membrane impregnated with a chromatographic stationary phase is another method of overcoming the poor concentration limits of detection. The schematic of the membrane preconcentration CE is shown in Fig. 13A. A piece of membrane is sandwiched between a short piece of fused silica tubing, and the CE capillary is coated with polybrene. Dilute samples are loaded first on to the membrane, where they are concentrated. Concentrated samples are washed, eluted onto the CE column, and separated under high voltage. Dilute mixtures of drugs, peptides, and proteins have been analyzed by this technique [100, 101].

Partially packed CE capillary

Packing the inlet end of the CE with C_{18} (or various other affinity packing materials) 1–5 mm in length (Fig. 13B) allows for on-line pre-concentration of dilute samples or the retention of compounds with specific groups, such as

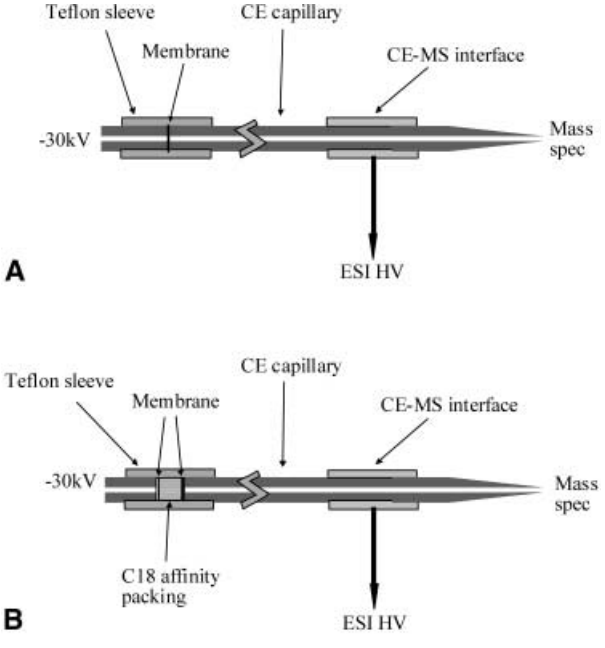

Fig. 13A, B Schematic of the membrane preconcentration CE (**A**), and on-line pre-concentration using partial packing technique (**B**)

phosphate. On-column packing provides a means of sample concentration without adding dead volume to the system, and can be combined with a sheathless interface for maximum sensitivity. This technique has been applied to the analysis of protein digests and to the selective retention of phosphorylated peptides in complex mixtures using an immobilized metal-ion affinity chromatography packed capillary [102]. However, because of the increased backpressure and decreased hydrodynamic flow within the separation capillary, and because a relatively large volume of organic solvent is needed to elute the analyte of interest, the solid phase packing technique significantly deteriorates the CE separation efficiency.

CE-MALDI MS analysis

While the focus of this article has been on the CE-MS analysis of complex mixtures using ESI, other ionization techniques, such as fast atom bombardment and, more recently, MALDI MS, have also been used in conjunction with CE. Both off-line and on-line CE-MALDI MS has been applied to the analysis of complex biological mixtures [103, 104]. While MALDI can directly analyze protein and peptide mixtures without separation, the mixtures are usually cleaned in order to remove salts, detergents, and other contaminations prior to analysis. Since ion suppression can significantly compromise the performance of MALDI at trace levels, separation of contaminants from the analyte of interest prior to MALDI analysis is desirable. Peptide mixtures have been detected at low-femtomole levels with high separation efficiency using on-line CE-MALDI-TOF MS, which utilizes a vacuum deposition of analytes on a moving belt [104].

Nucleic acids

Both CE and CITP, in conjunction with ESI-MS, have been applied to the analysis of nucleotides [105, 106]. More recently an overview article on the application of CE/ESI-MS in the identification and quantification of modified nucleosides, nucleotides, and oligonucleotides has been published [107]. Since investigation of DNA adducts requires a detection of ~1 adduct in ~10^6 unmodified nucleobases in healthy individuals, and considering that approximately 150 fmol of carcinogen-DNA adducts is present in 1 g of wet tissue, a highly sensitive technique with a high degree of separation efficiency was required for identification. The separation of the modified oligonucleotides was achieved by adding a linear polymer (N-vinylpyrrolidone) to the buffer solution as a sieving matrix. The physical network formed by the linear polymer acted as a pseudo-phase and allowed separation on the basis of hydrophobic interactions. The resolved analytes were identified by using ESI-ion trap-MS [108].

Microfabricated and multi CE/ESI-MS

Recently, high-throughput microfabricated devices designed for the MS analysis of a large number of compounds in a short period of time have received considerable attention. Several techniques can be used to couple microchip to MS using ESI, including: flat edge surface [109, 110, 111], attachments of ESI emitter tips directly to the exit of the microchips by a double etching procedure [112, 113], polymer casting [114], hand drilling [115, 116], or microfabricated ESI tips [117, 118]. Some of these ESI-MS interface techniques have been applied to CE/ESI-MS analysis [112, 113, 115, 116].

To increase the CE/ESI-MS throughput, several strategies have been introduced. One uses a microfabricated CE/ESI-MS in conjunction with automated sampling from a microwell plate [119]. Our strategy, however, is to use a multi-CE capillary in conjunction with our multi-inlet TOF MS [120]. In the latter design, eight separations are carried out at the same time in eight CE capillaries interfaced to an eight-nozzle TOF MS utilizing the split-flow interface design.

Future direction

-1. Analysis of single intact cells by CE-MS looks very promising. To detect the chemical components of a single cell with a quantity of less than 50 amol, however, the sensitivity of present mass spectrometers must be enhanced. Reduction of chemical background noise, or using ultra high-resolution mass spectrometers that can separate analytes of interest from the background noise, can improve detection limits for single cells.
-2. The combination of preconcentration techniques in conjunction with CE-MS will enable detection of minor components of cells using whole cell lysates of many cells.
-3. CE-MS analysis of whole cell digests using ultra high mass accuracy of modern high field (>9 Tesla) FTICR MS.
-4. High-throughput analysis of chemical and biological compounds using multiple CE capillaries (conventional capillaries or CE-on-chip) using single nozzle or multiple nozzle mass spectrometers.
-5. Commercialization of ultra high voltage CE (>30 kV), and further development of multi-electrode CE for ultra high voltage and ultra high resolution CE-MS. The latter was recently demonstrated by using dual in-capillary electrode CE/ESI-MS [121].

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