Capillary Electrophoresis coupling to Mass Spectrometry (CE-MS), an advanced technique orthogonal to LC-MS for high resolution separation and accurate molecule identification

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Capillary Electrophoresis (CE) coupled with Mass Spectrometry (MS) has been under investigation for the past couple of decades[1]. Combining the high separation power of CE with MS promises to yield more sensitive detection and higher information content on the analytes than current methods such as standard UV detection. Since those early days, there have been many improvements and technology advancements on CE and MS instruments, as well as the interfacing devices. Modern Time of Flight (TOF) MS instruments and robust commercial interfaces provide increasingly sensitive routine analysis with accurate mass data.

The basic approach to CE-MS is to use Electrospray Ionization (ESI) techniques. The advantage to this system is that typically ionized or polar compounds separated by the CE under low flow conditions (low nl/min range) can then be efficiently transferred from the liquid phase into gas phase ions required by MS instruments. In addition, ESI does not decompose fragile molecules, like proteins.

Routine CE-MS requires the use of volatile buffers or low concentration salts. This avoids contamination of MS instruments and the suppression of analyte ions by salts competing for entrance to the MS. Separation by CE and ionization of molecules in the ESI source are both based on analyte chemistry and require optimization for best results. It is critical to choose the correct solvents and pH ranges for solubility, to allow for optimal CE mobility and for high ionization efficiency during desolvation. This may call for different chemistries for the separation buffers and for a sheath liquid controlling the ESI processes.

High resolution in CE provides narrow peak widths, often just a few seconds wide, that require rapid scanning by the MS if full spectra are needed. For this reason TOF-MS is often the chosen tool providing sufficient data points over a peak, yet still offering full mass range. Since TOF or Quadrupole-TOF (allowing specific preselection of ions and MS/MS fragmentation capabilities) can have very high resolution (e.g. 40.000 resolving power), exact masses and isotopic relations can be determined leading to molecule identification.

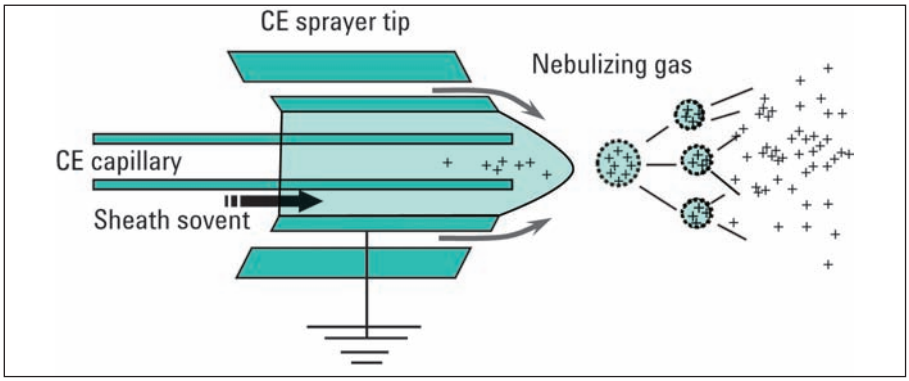

Figure 1. Sheath Liquid Interface for CE-MS as triple tube setup. The inner tube is the fused silica CE-capillary followed by grounded stainless steel (or platinum) tube guiding sheath liquid from an LC pump or other device. The nebulizing gas used for enhanced droplet formation is guided through the outer tube.

Why use CE or CE-MS? CE is an orthogonal method to HPLC where separation is based on mobility of ions in an electrical field, instead of chromatography. Intrinsically, CE can handle ionized or very polar compounds best, which are often difficult to separate on a LC. CE-capillaries are usually fused silica based open tubes with a 25-100 µm inner diameter. Adsorbance of compounds such as proteins to the inner wall is less critical compared to HPLC columns. This is due to the much larger surface area of HPLC packing materials and the risk of getting physically stuck. The adsorbance effects to fused silica in CE can be counteracted by using specifically coated capillaries. This could be permanently bound coatings (e.g. polyvinyl alcohol) or various dynamic coatings with cationic or anionic modifiers added to the liquid phase. Hence, CE may also be used as a separation tool for large polymers or biological compounds, like proteins or nucleic acids.

Interfaces for CE-MS need to accomplish various tasks: position the CE capillary physically close to the MS entrance, provide electrical contact to the free end of the CE capillary and support the right droplet formation needed for desolvation and creation of free gas phase ions.

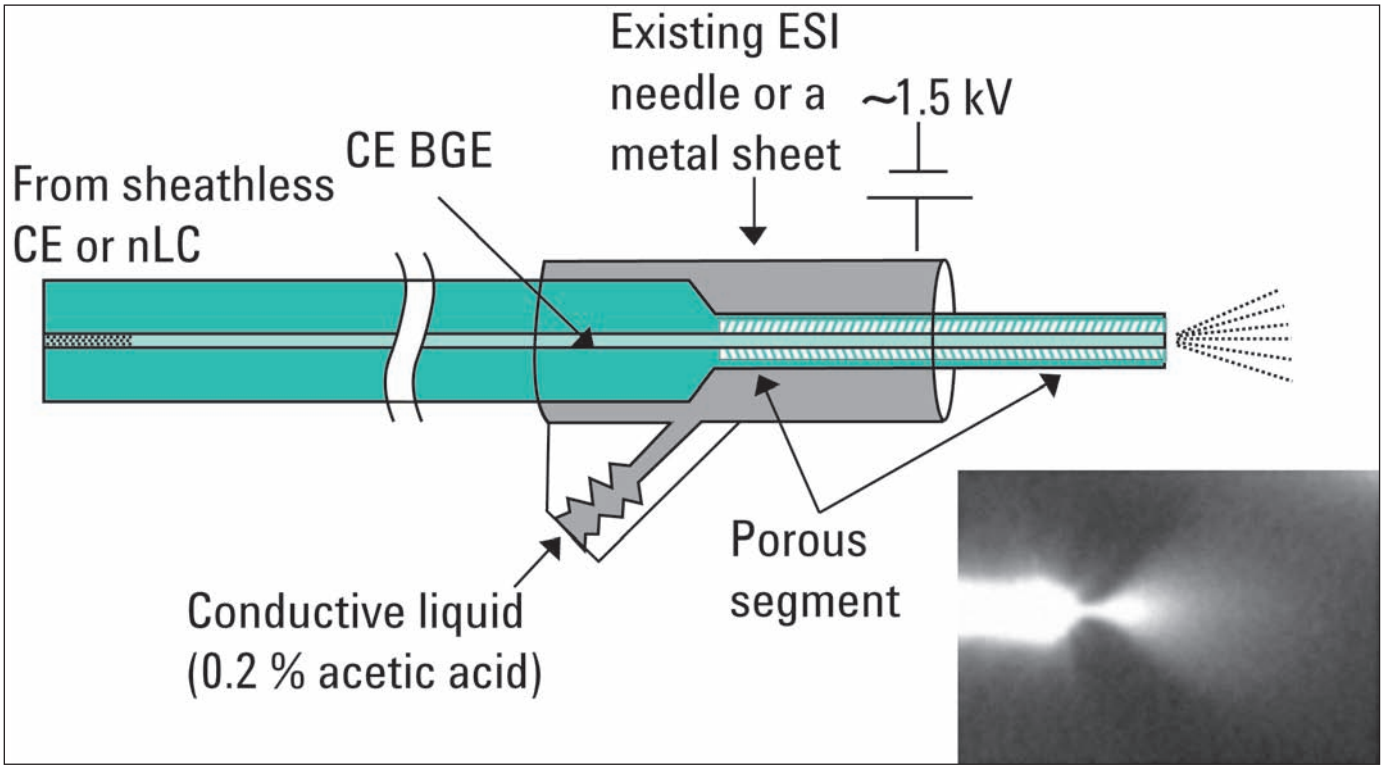

Figure 2. Sheathless flow CE-MS interface as presented by Mehdi Moini[2] in 2007.

Currently, the most used interface technique is a sheath liquid approach where a make-up flow of 1-5 µL/min is added via a pump (LC or syringe device). In an adjustable triple tube needle (see Figure 1), the CE capillary is combined with the sheath liquid and a nebulizing gas flow. This needle unit then enters into the MS ion source. Performance of this setup is very robust as there is a constant flow rate for the MS granted by the sheath liquid. Also, the chemistries for separation and ionization can be decoupled efficiently by adding acidic or basic sheath liquids with organic contents to an aqueous buffer solution. Ideally, the capillary end is set on ground potential to avoid difficulties connecting to the ion source and its high voltage requirements.

Another option is to use sheathless interfaces (see Figure 2) which are currently in development[2]. This approach can avoid the dilution effect by the sheath liquid and result in improved sensitivities. In this process, CE nanoliter flowrates are provided directly to the MS. As there is only one background electrolyte for separation and ionization this must be optimized accordingly to fulfill both needs.

There is a broad field of interest for CE-MS with accurate mass detection applications ranging from food, forensics, and biotechnologies to basic research in biology, such as in metabolomics. Metabolomics is a good example to show the breadth of CE-MS capabilities. The combined performance of CE and MS allows the detection and analysis of organic acids and very polar small molecules in a complex biological and sometimes sample limited situation. An example for such a metabolomics analysis using CE-TOF MS is shown in Figure 3. CE provides a fast and robust separation of this complex mixture requiring minimal or no sample preparation. TOF-MS offers very fast and full range spectra with an extraordinary mass accuracy. There are currently many groups in metabolomics research using this approach[3], and commercial offerings for industrial requirements also exist.

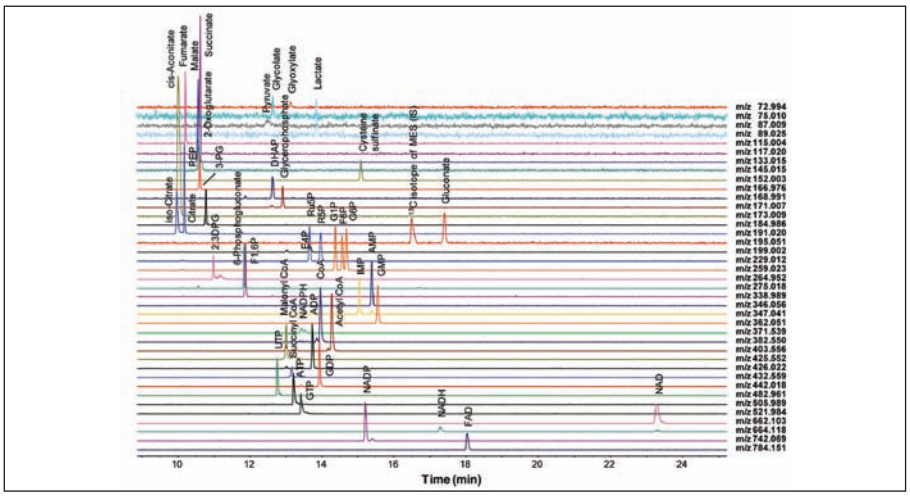

Figure 3. CE-TOF MS ion electropherograms for a standard mixture of anionic metabolites in the components of glycolysis, pentose phosphate and the TCA pathways (Soga et al.[4]).

In summary, CE-MS has evolved into a reliable system suitable for industrial applications. It has become a standard analytical tool for applications not ideally suited for LC-MS (e.g. ionic, very polar or very large compounds). Via ESI, a portfolio of MS options are now available, ranging from simple Quadrupole and Ion trap instruments to Triple-Quadrupole, TOF or Q-TOF systems. For small and less polar compounds or suppressing buffer conditions, there are other promising ionization methods available, such as Atmospheric Pressure Chemical Ionization (APCI) or Atmospheric Pressure Photo Ionization (APPI).

References

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2) *M. Moini Anal. Chem. 79 (2007) 4241*
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